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THE GROWTH OF OYSTERS PARASITIZED BY THE FUNGUS *DERMOCYSTIDIUM MARINUM* AND BY THE TREMATODE *BUCEPHALUS CUCULUS**

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Although there are many publications on the parasites of oysters, until recently there have been none on the effects of these parasites on growth. Indeed, published records of the effects of parasites on the growth of animals of any kind still seem rare enough to make any new record worth publishing. In this paper, we present data on the growth of parasitized and uninfected oysters of known age, under natural conditions, over a period of 16 months. The results of our field experiment are supplemented by data from a laboratory experiment by Dr. Willis G. Hewatt. The oyster used in the experiments was *Crassostrea virginica* (Gmelin, 1790), the commercial oyster of the Atlantic and Gulf coasts of North America. More than half of the oysters in both experiments developed infections of the parasitic fungus *Dermocystidium marinum* Mackin, Owen, and Collier, 1950. The numbers of fungus-infected and of uninfected individuals were sufficient to provide a good test of the effect of *Dermocystidium marinum* on the growth of oysters. One oyster, otherwise uninfected, was parasitized by sporocysts of the trematode *Bucephalus cuculus* McCrady, 1874. No conclusions can be based on this one specimen, but the record of its growth is presented for comparison because it is unique. Apparently the only previous report on the growth of a trematode-infected oyster is that of Hoshina and Ogino (1952), which was concerned with metacercariae, not sporocysts, and did not involve castration of the host as in the case of *Bucephalus* infection.

METHODS

A growth experiment was carried out during the period May, 1948 to May, 1950, at the Bay Sainte Elaine oil field operating camp of The Texas Company in Terrebonne Parish, Louisiana. This camp is built on pilings in a waterway connecting Bay Coon Road and Bay Sainte Elaine, small tidal water bodies in the salt marsh on the west side of Terrebonne Bay. It is five or six miles inland from the Gulf of Mexico and is located at approximately 29°10′ north latitude and 90°40′ west longitude.

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The salinity and the temperature of the water at the site of the equipment were measured each morning, at approximately 7 a.m., during the first year of the experiment (May 1, 1948 to May 22, 1949), and during most of the preceding year. The salinity ranged from 13 to 26 parts per thousand; the median for the period was approximately 19. Water temperatures ranged from a winter minimum of 5°C to a summer maximum of 31°C; in 1948 they were above 20°C almost continuously from April 1 to November 1, and were above 25°C during most of that period. Water temperatures below 10°C occurred only after the passage of cold fronts or "northers" and usually lasted only a few days, even in midwinter. Temperatures below 10°C were recorded on 25 days in 1947–48 and on 5 days in 1948–49. The winter mean (December–March) was 13.9° in 1947–48 and 16.5° in 1948–49.

On May 1, 1948, a number of 4 by 3 inch pieces of "roofing tin" (thin galvanized sheet steel) were placed in the water to catch oyster spat; that is, so that oyster larvae would attach themselves on the surface of the metal. On June 26 the "tin" plates, with young oysters (spat) attached, were secured in a vertical position in three heavy trays (numbered 2, 3, and 4), which were suspended in the water under a tank battery platform and oil loading dock. All but one young oyster had been scraped off of each side of each plate. Each plate was marked by a number painted on the metal. All of the young oysters were measured in two dimensions (length and height) once each month thereafter. However, many of the original spat died, or fell off the plates and were lost, and these were replaced by younger spat which had attached to the plates during the period from June 26 to October 28, 1948. On December 20, 1948, all of the young oysters were removed from the plates, and each surviving oyster was marked by a number painted on its shell. On January 23, 1949, and once each month thereafter, each oyster was weighed individually, and three dimensions (length, height, and width or thickness) were measured with vernier calipers. From June 20, 1949, to May 9, 1950, all of the measurements were made by Mr. T. Jack Clark.

Tray No. 4 was lost in a storm in September, 1949. Tray No. 2 was pulled up by some person and left on the dock so that all of the oysters were killed by exposure sometime between November 2 and December 1, 1949. Tray No. 3 continued to be examined monthly until May 9, 1950, when the experiment was terminated. The original oysters were 24 months old on May 9, 1950; some of the replacements added between June 26 and October 28, 1948, were one, two, or three months younger, but these had grown as large as the original ones.

The shells of all oysters in tray No. 3 were examined carefully. No evidence of mechanical injury or of damage to shells by predators was noted during the period of record. By the end of this period, all of the oysters had moderate to heavy infestations of shell-boring annelids or "mud worms," *Polydora websteri* Hartman, and all had "mud blisters" on the inside surface of the shell, caused by *Polydora* penetrations. Two had very light infestations of boring sponges, *Cliona* sp., and 12 had very light infestations of boring clams, *Martesia smithii* (Tryon). Only one oyster shell was penetrated to the inner lining by *Cliona*, and only one was completely penetrated by *Martesia*. Inspection of the data did not reveal any indication of a possible correlation between shell-borer infestations and the different growth rates of individual oysters.

The meats of the 23 survivors were fixed on May 9, 1950, and were later sectioned and stained for parasitological study. The sections were examined by Dr. J. G. Mackin, who had never seen the growth records. Dr. Mackin rated the *Dermocystidium* infections as light, moderate, and heavy, or in some cases "light to moderate" or "moderate to heavy." In order to simplify the tabulation of data, we reclassified the infections, on the basis of Dr. Mackin's notes, as "light" and "heavy," the "light" class including all which were not diagnosed as "heavy" or "moderate to heavy." One slide had been lost, and one was stained so lightly that Dr. Mackin could not use it. Of the 21 oysters studied, eight were uninfected, one had sporocysts and cercariae of *Bucephalus cuculus*, nine had "light" infections of *Dermocystidium marinum*, and three had "heavy" infections of *D. marinum*. A few spores of *Nematopsis* sp. were seen in 17 oysters.

RESULTS

Tables 1 and 2 show the growth records for three groups of survivors: negative or uninfected oysters, oysters lightly (or moderately) infected by *Dermocystidium*, and oysters heavily infected by *Dermocystidium*. Table 1 shows, for each of the

TABLE 1.—*Weight in grams of the heaviest individual, the lightest individual, and the average individual in each of 3 classes of oysters: negative, lightly infected, and heavily infected (by* Dermocystidium marinum*), on each of the 16 monthly examination dates*

Date weighed	Maximum weight			Minimum weight			Mean weight		
	Neg.	Light	Heavy	Neg.	Light	Heavy	Neg.	Light	Heavy
1, 23, 49	36	52	56	11	10	12	24	26	36
2, 20, 49	40	59	59	14	12	16	29	30	39
3, 19, 49	48	67	63	18	14	20	34	35	42
4, 19, 49	56	77	65	23	16	25	40	40	45
5, 20, 49	68	92	69	29	21	32	49	48	49
6, 20, 49	88	108	79	39	29	44	62	61	58
7, 20, 49	98	124	89	48	35	51	73	72	65
8, 20, 49	111	138	99	58	39	55	85	83	72
9, 30, 49	121	160	109	70	50	56	100	99	80
11, 2, 49	144	179	114	82	56	56	112	111	86
12, 1, 49	160	196	119	89	59	59	123	122	93
1, 4, 50	187	219	125	100	61	61	139	136	99
2, 1, 50	207	240	126	108	66	61	154	150	102
3, 9, 50	230	259	130	118	72	61	169	162	106
4, 11, 50	250	276	140	123	78	61	181	173	109
5, 9, 50	270	282	141	127	79	61	189	182	110

16 months, the weight of the heaviest individual (maximum weight), the weight of the lightest individual (minimum weight), and the mean weight of all individuals in each of the three classes: negative, light, and heavy. Similarly, Table 2 shows the length of the longest and the shortest individual in each class, and the mean length of all individuals in each class, on each of the 16 monthly examination dates.

Figures 1 and 2 show the data from Tables 1 and 2, respectively, in the form of graphs, mean size (weight or length) against time. The measurements of the one oyster infected by *Bucephalus* are also plotted on each graph for comparison.

The weight measured in this experiment was the weight of the live oyster in air with the shell closed and containing all of the sea water which was inside of the shell before it was removed from the water. (All extraneous matter and attached organisms had been scrubbed off the outside of the shell.) The shells were later weighed after the meats and water had been removed on May 9, 1950. At this time the shell weights were 73% to 85.5% of the total weights, average 79.5%. It might be argued that we were actually measuring rate of shell deposition rather

than growth, but that argument could be made, with almost as much justification, against the use of height or weight as an index of size in studying the growth of children, since non-living bone material makes up almost all of the height and a considerable part of the weight. At any rate, live weights and shell measurements are the indices of size which have always been used by the oyster biologists, and experience has proved the increase in shell size to be a good indication of growth.

The length measured in this experiment was the length of the right valve of the shell, from the umbo on the extreme anterior end to the farthest point on the posterior edge of the shell. Length was chosen as an index of size (in preference to height, thickness, or the product of all three dimensions) because previous experience had shown that length is the first dimension to be affected by adverse conditions. When an oyster begins to suffer from a disease or other adverse condition, it first stops adding new shell material at the posterior edges of the valves; the mantle tends to retract, so that new shell material is deposited inside the edges of the valves rather than beyond the edges as in oysters which are growing normally. Thus

TABLE 2.—*Length in millimeters of the longest individual, the shortest individual, and the average individual in each of 3 classes of oysters: negative, lightly infected, and heavily infected (by* Dermocystidium marinum*), on each of the 16 monthly examination dates*

Date measured	Maximum length			Minimum length			Mean length		
	Neg.	Light	Heavy	Neg.	Light	Heavy	Neg.	Light	Heavy
1, 23, 49	59	58	66	36	37	38	49	47	53
2, 20, 49	64	63	67	40	40	40	53	50	53
3, 19, 49	65	65	67	41	41	41	55	53	54
4, 19, 49	67	65	67	43	41	43	56	54	55
5, 20, 49	74	71	69	45	44	49	62	58	58
6, 20, 49	77	75	71	51	49	54	66	62	60
7, 20, 49	79	77	71	54	51	57	69	65	62
8, 20, 49	84	84	75	57	52	60	73	69	66
9, 30, 49	86	83	79	65	58	59	77	73	69
11, 2, 49	91	92	78	69	61	61	80	76	70
12, 1, 49	94	93	80	70	62	62	83	78	73
1, 4, 50	98	95	80	71	60	62	86	82	73
2, 1, 50	99	98	80	72	63	62	87	84	73
3, 9, 50	103	101	80	75	63	61	91	87	73
4, 11, 50	105	102	82	77	64	61	94	88	74
5, 9, 50	107	103	82	77	63	60	95	89	74

an oyster may continue to gain in thickness and weight after it has stopped increasing in length.

In the present experiment, the uninfected oysters gained 12 mm. in average length and 66 grams in average weight during the last five months, December 1 to May 9. The three oysters with heavy *Dermocystidium* infections gained only 1 mm. in average length during the last five months, but increased 11 grams in average weight. The average weight of the lightly infected group was practically identical with that of the uninfected group for the first 11 months, and was only slightly less than that of the uninfected group during the last five months, but the average length was less than the average length of the uninfected group throughout the last 15 months. The single *Bucephalus*-infected oyster did not gain in length (or in height) during the last two months, while the weight continued to increase because of a 3 mm. increase in thickness. These seem to be examples of the phenomenon described in the last paragraph.

The general picture seems clear enough: the uninfected oysters continued to grow at an almost constant rate throughout their second year of life, while the oysters with light *Dermocystidium* infections lagged slightly in growth, especially

in the last five months, and the oysters with heavy *Dermocystidium* infections grew more and more slowly and finally came to a complete stop. The significance

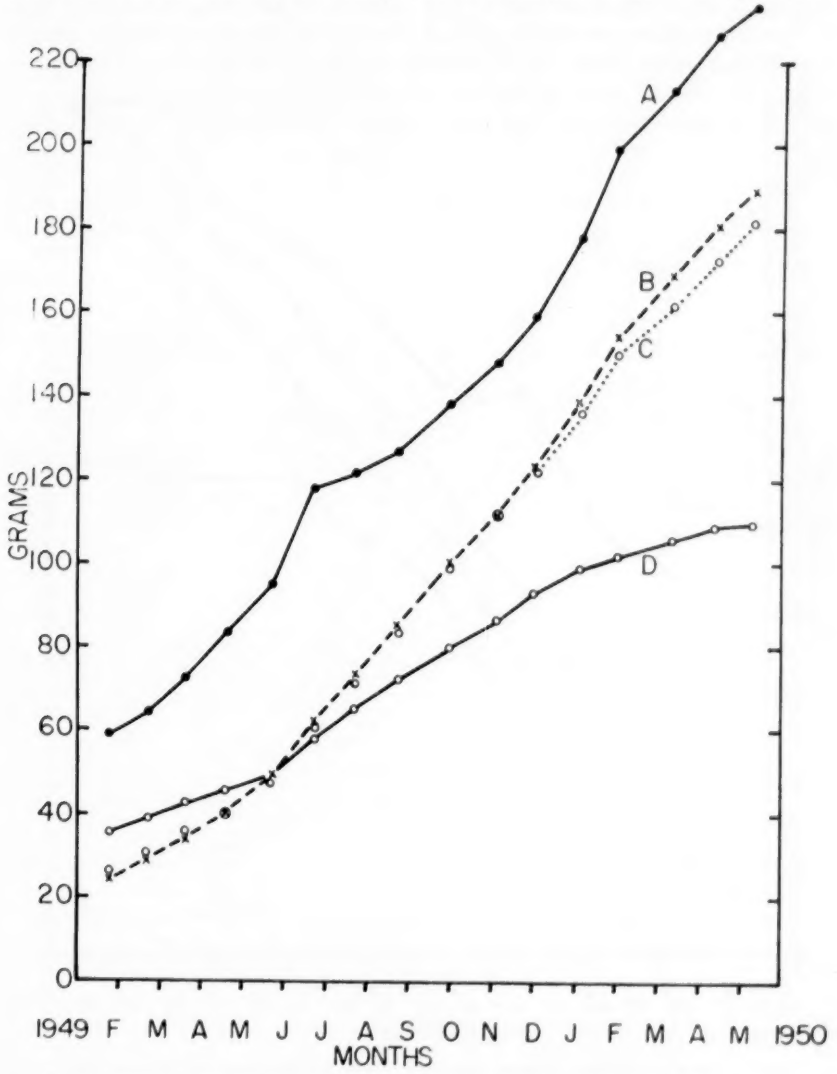

FIG. 1. Growth of four groups of oysters as shown by observed weight of live oysters at each monthly examination during the 16-month period January 23, 1949, to May 9, 1950. A, oyster with *Bucephalus* sporocysts; B, average of eight uninfected oysters; C, average of nine oysters with light infections of *Dermocystidium*; D, average of three oysters with heavy infections of *Dermocystidium*.

of the data on the single *Bucephalus*-infected oyster is not certain. It was an unusually fast-growing individual throughout its first year of life, up to June 1949, and then its growth began to lag slightly behind that of the uninfected group;

the failure to gain in length or height during the last two months may have been caused by the parasite.

Dr. M. J. Garber, biometrist of the Genetics Department, A. & M. College of Texas, has made a statistical study of the data on the oysters heavily infected by *Dermocystidium*, those lightly infected, and those uninfected. He compared

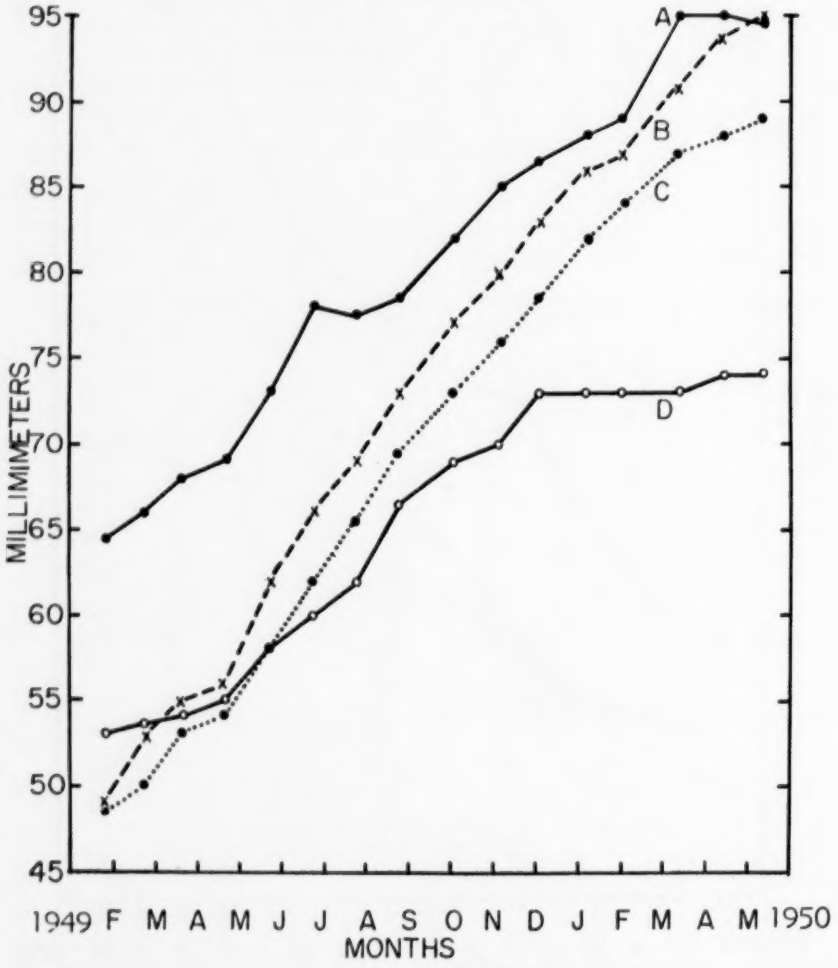

FIG. 2. Growth of four groups of oysters as shown by observed lengths at each monthly examination during the 16-month period January 23, 1949, to May 9, 1950. Legends as in Fig. 1.

the average lengths and the average weights of the three groups of survivors at each monthly examination, and also the slopes of the growth curves, and found in each case that the differences between the three groups are real. There is less than one chance in one hundred that these differences were due to chance or accidental coincidence.

The oysters which died during the period of the experiment were not examined for parasites. Since the oysters were inspected only once a month, the meats were either decayed or completely gone by the time the deaths were discovered. However, growth records are available for each individual which died. During the 16-month period of record, 23 oysters died in tray No. 3, and 14 of these (61 per cent) had stopped growing at least one or two months before death. In the last 10 months, 11 (or 73%) of the 15 which died had stopped growing for at least

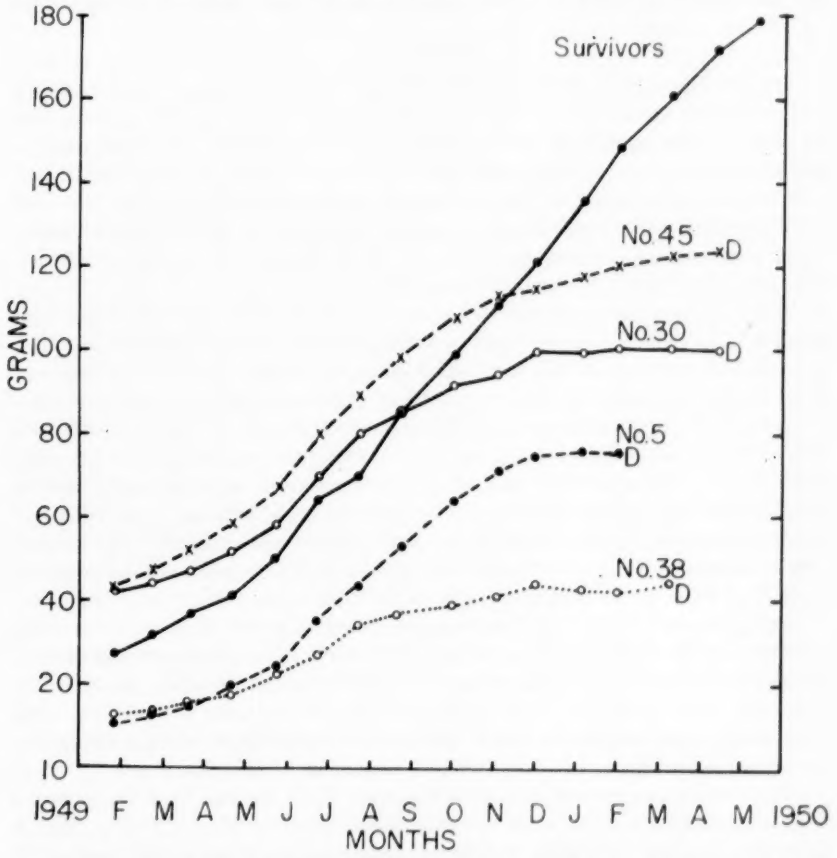

FIG. 3. Growth of the 23 oysters which survived to the end of the experiment and of the four oysters which died during the last two months, as shown by observed weights of live oysters at each monthly examination, January 23, 1949 to May 9, 1950.

the last one or two months before death. All of the four which died during the last two months had stopped growing at least two or three months before death (See Figure 3.) Of the 11 oysters which died in tray No. 3 during the last three and one-half months, 8 had stopped gaining in either weight or length, or both, some time before death. Similarly, 10 of the last 13 oysters which died in trays No. 2 and No. 4 had ceased growing for some time before death. Some of the

oysters which died had not gained appreciably, or had even lost in weight or length, or both, during the last six or seven months preceding death. Figure 3 compares the increase in weight of the last four oysters which died in tray No. 3 with the increase of the 23 oysters which survived to May 9, 1950.

All of the oysters in tray No. 3 whose growth had ceased finally died with the exception of four. All of the four non-growers which were still alive on May 9, 1950, had *Dermocystidium* infections: two heavy and two light. It seems probable that these four would have died if the experiment had been continued a little longer.

DISCUSSION

The results of this experiment indicated that *Dermocystidium* retarded growth in proportion to the intensity of the infection, and that oysters which stopped growing died. These results did not establish a direct relationship between infection and death, because the oysters which died were not examined for parasites. However, a direct relationship between *Dermocystidium* infection and death had been established by another experiment, conducted by Dr. W. G. Hewatt in 1949 while working on another phase of the Texas A. & M. Research Foundation's Project Nine (Hewatt, 1953, and unpublished data).

In Dr. Hewatt's experiment, 28 oysters were kept in separate fingerbowls supplied with running water pumped from Barataria Bay. One group of 14 oysters was supplied with water which had passed through a settling tank and a fine Monel metal screen to remove all but the smallest plankton organisms, and the other group received unfiltered bay water. Oysters which died were replaced by oysters from a tray suspended in the bay. Each of the 28 oysters was weighed under water once a week. Oysters which died during the experiment were promptly fixed in Zenker's fluid and later sectioned. At the end of the experiment, a small piece of mantle was clipped from each surviving oyster, fixed, and sectioned. All sections were examined by Dr. J. G. Mackin, who found heavy *Dermocystidium* infections in all 25 of those which died during the experiment, while only 11 of 31 survivors (including some from the tray) were infected, and only one of these had a heavy infection. In this experiment, as in our own, nearly all of the oysters which died had stopped growing or (in most cases) had lost weight for some weeks before they died, while nearly all of the survivors continued to gain weight each week. Also, nearly all of the oysters which had ceased to grow finally died, while all but one or two of the "growers" were still alive at the end of the experiment.

Dr. Hewatt's experiment ran from February 11 to August 19, 1949, a period of approximately 6 months, while our experiment ran from May 1, 1948 to May 9, 1950, with records on weights and linear measurements of individuals during the last 16 months. Dr. Hewatt weighed his oysters under water once a week, while we weighed ours in the air once a month. His experiment was conducted under laboratory conditions and ours under field conditions. Nevertheless, the two experiments produced very similar results, both showing a close correlation between slow growth and death. In addition, Dr. Hewatt's experiment showed a direct relationship between heavy *Dermocystidium* infection, cessation of growth, and death on one hand, and light or no infection, continued growth, and survival on the other hand.

Mackin (1951) has described the histopathology of *Dermocystidium marinum*

infections in oysters. Ray, Mackin, and Boswell (1953) have reported quantitative measurements of the effect of this fungus. The parasites invade all tissues and digest or dissolve tissue elements around them so that the oyster (the living body or meat) shrinks and becomes lighter until it finally dies. Apparently no toxin is produced. In advanced stages of infection the shell-secreting function of the mantle is damaged and the mantle shrinks away from the outer edges of the shell, so it is easy to understand why growth is reduced or stopped and why a heavily infected oyster may become progressively shorter and lighter until it finally dies. Most of the heavy infections develop in warm months, and oyster mortality is confined almost entirely to the warmer months in Louisiana waters. Unfortunately, the warm season (with water temperatures above 20°C) lasts more than half the year on the Gulf Coast.

The effects of *Bucephalus cuculus* infection are not so well known. From personal observation on many *Bucephalus*-infected oysters, we know that the sporocysts are confined to the gonads in early stages of the infection. The gonad tissues are eventually destroyed and replaced by the mass of branching sporocysts, so that oysters with well-developed infections never produce any eggs or sperm cells. We have been able to confirm the statement by Tennent (1906) that "at this stage the oyster may still appear plump and healthy." It seems possible that as long as the sporocysts are confined to the gonad region, a *Bucephalus* infection may cause increased growth of the oyster in the same way that other gonad-destroying trematodes increase the growth of snails, as described by Rothschild (1936, 1938, 1941a, 1941b). However, Tennent (1906) went on to say that "in the last stages the tissues of the oysters seem almost completely wasted away." We have not been able to confirm this statement by personal observation, but it is probably true, for we have observed that after completely replacing the gonad the sporocysts spread out into the mantle, the gills, the digestive gland, and even into the adductor muscle. Such a widespread invasion of the tissues could hardly fail to interfere with the normal functions and cause a diseased condition. Possibly the one *Bucephalus*-infected oyster in our experiment was beginning to feel the harmful effects of an advanced infection. Two of Dr. Hewatt's oysters had *Bucephalus* infections, but these did not yield any useful information because they both died with heavy *Dermocystidium* infections.

Incidentally, *Dermocystidium marinum* seems to be harmless to man, and *Bucephalus cuculus* is actually beneficial from the gastronomic standpoint. In southern waters, normal oysters spawn so heavily and so long that they become emaciated and nearly tasteless by late summer, but *Bucephalus*-infected oysters, being castrated or "caponized," remain fat and retain an excellent flavor all summer. Personal tests have proved that the *Bucephalus* sporocysts taste even better than the oyster.

For further information on *Bucephalus cuculus*, see Hopkins (1954).

SUMMARY

The authors studied the growth of Louisiana oysters of known age under field conditions during the 16-month period January 23, 1949, to May 9, 1950. Linear dimensions and live weights of individual oysters were measured monthly. Twenty-one survivors were examined for parasitic infections.

In a laboratory experiment conducted in the summer of 1949 by Dr. W. G.

Hewatt, the 25 oysters which died as well as the 31 survivors were examined for parasites. Each oyster was weighed weekly.

In these two growth experiments, most of the oysters which died had stopped growing several weeks before death, and nearly all individuals which stopped growing died before the end of the experiment, while the oysters which had not stopped growing survived. Cessation of growth and death were associated with heavy infections of the fungus *Dermocystidium marinum* Mackin, Owen, and Collier. The one oyster which was infected by sporocysts of the trematode *Bucephalus cuculus* McCrady was an unusually fast grower until the latter months of the experiment, and then lagged behind the uninfected oysters. The uninfected oysters continued to grow at an almost constant rate.

The authors conclude that under Louisiana conditions *Dermocystidium marinum* usually causes a slowing of the growth of infected oysters, and finally a complete cessation of growth, ending in death. It is suggested that *Bucephalus cuculus* may cause an increase in growth rate during the early stages of infection, and a slowing of growth during later stages when the sporocysts spread from the gonad to the gills, mantle, and muscle. Uninfected oysters, in Louisiana waters, continue to grow at an almost constant rate at least through their second year of life. Inclusion of parasitized individuals in a growth experiment may lead to erroneous conclusions if the growth curves are interpreted as "normal."

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A PUNCHED CARD SYSTEM FOR FILING PARASITOLOGICAL BIBLIOGRAPHY CARDS

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Anyone who has attempted to keep a comprehensive card-file of abstracts or titles of papers in his scientific field knows that as the file becomes larger it also becomes clumsier. Information in it is lost simply because of the great amount of time which would be required to dig it out. In the field of parasitology, for example, one can file his cards either by parasite genus or species, by subject, by host, or alphabetically by author. The last system is unsatisfactory by itself for large files, but is usually employed in combination with one of the other systems.

Such files suffer from two principal disadvantages. The first is that in many papers more than one parasite, subject or host is discussed, so that many duplicate or cross-reference cards must be used. The second and even more serious disadvantage is that when one system is selected for a card-file, other systems are largely excluded. As a result, the file is much less valuable than it otherwise might be. For example, in a file arranged taxonomically by parasite groups and genera, one can quite readily find abstracts or papers on any particular parasite. However, if he wishes to find papers on the chemotherapeutic effects of certain drugs on all parasites against which they have been used, he must go through the whole file card by card. He must do the same if he wishes to find papers on all parasites of a particular host animal. The only way to avoid this would be to maintain three complete duplicate files. This is not usually feasible.

The use of punched cards obviates many of these difficulties, and produces a much more flexible and usable file. These cards are provided with a series of holes around their edges which can be punched out in various combinations. Once a code has been worked out for these combinations, the cards can be sorted into groups by passing a long needle through a stack of them and allowing the punched ones to drop out. Much information on punched cards and their uses and applications is given by Casey and Perry (1951).

A special code must be worked out for each application, and it is the purpose of this paper to present a code which has been developed for general, veterinary and medical parasitology.

To be of greatest use, a card file should contain not only the authors, titles, dates and journal citations of the papers in it, but also brief abstracts. The most convenient and complete method, and the least time-consuming and expensive, is to clip the abstracts from a standard abstracting journal and paste them on the cards. This requires that two copies of the journal be purchased, but the additional expense is minor. Abstracts from *Biological Abstracts* make an excellent backbone for a card file. A second copy of a section of this journal may be purchased for clipping and pasting without the indices for approximately half the cost of the first copy.

Cards are prepared by clipping abstracts and attaching them to punched cards. Mucilage, library paste or mounting tissue can be used for this purpose. I use

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mucilage, and apply it only along the top of the abstract. If an abstract is too long to fit on the card, the bottom can be folded under, or the excess portion can be cut off and pasted on the back of the card.

Punched cards are available in several sizes. After an initial trial of 5 by 8 inch cards, it was decided that 3 by 5 inch cards were more practical and convenient for a personal card file. It is seldom necessary to use more space than is available on them, and they are less bulky and cheaper than the larger cards. Since the small cards have only 57 holes in comparison with the 97 holes of the 5 by 8 cards, the number of combinations available for use is more limited. However, it has been found during the preparation of several thousand cards that this is not a serious

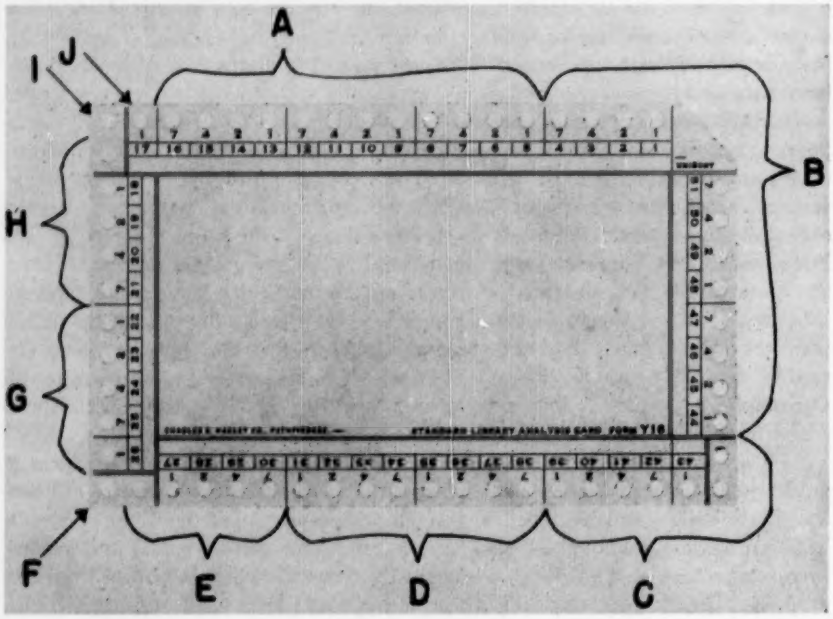

FIG. 1. Blank punched card for parasitology bibliography. A, first parasite genus (12 holes, decimal code, 999 numbers); B, second parasite genus (12 holes, decimal code, 999 numbers); C, unused, available for other information; D, host (8 holes, decimal code, 99 numbers); E, first author alphabetical code (5 holes, additive code, 30 numbers); F, more than 2 subjects; G, first subject (5 holes, additive code, 30 numbers); H, second subject (5 holes, additive code, 30 numbers); I, more than 1 host; J, more than 2 parasite genera.

objection, even though every parasite genus or even every parasite family cannot be given a separate number. The code as it stands has proven satisfactory for handling abstracts of veterinary, medical and general parasitology in several years' issues of *Biological Abstracts*.

CODING SYSTEM

Around the edge of each card are 12 groups or fields of four holes each, plus nine extra holes at the corners. The holes in each field are numbered 7, 4, 2 and 1, respectively (Fig. 1).

Two types of numerical code are used in punching out the holes. In the decimal system, the holes in each field of four are punched out in combinations which will

give numbers from 1 to 9. One field is used to represent units; the next one to its left, tens; the next one, hundreds, etc.

In the additive code, the holes in each field are punched out in combinations which will give numbers from 1 to 14. If a fifth hole is added, it is possible to code numbers from 0 to 29; this can then be used as an alphabetical code if desired. Each time an additional hole is added, the number of possible combinations is doubled.

In the present system (Fig. 1) a decimal code covering three fields of four holes each is used for the most important parasite genus discussed in the paper. This permits the use of 999 numbers in the code. A second set of three fields is used for a second parasite genus. A decimal code covering two fields and allowing

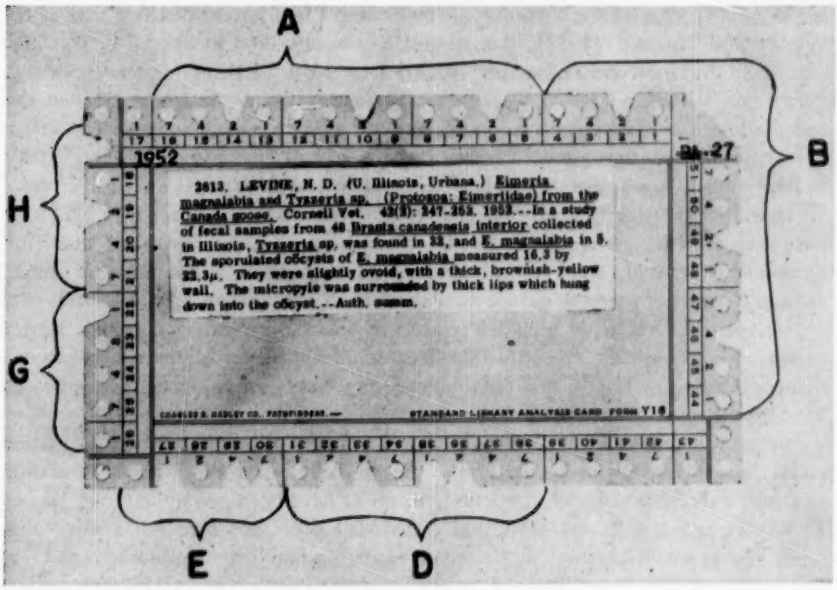
A
B
H
G
E
D
1952
2613. LEVINE, N. D. (U. Illinois, Urbana.) Eimeria magnalabia and Tyzzeria sp. (Protozoa: Eimeriidae) from the Canada goose. Cornell Vet. 43(3): 347-353. 1953.--In a study of fecal samples from 40 Branta canadensis interior collected in Illinois, Tyzzeria sp. was found in 33, and E. magnalabia in 8. The sporulated oöcysts of E. magnalabia measured 16.3 by 23.3μ. They were slightly ovoid, with a thick, brownish-yellow wall. The micropyle was surrounded by thick lips which hung down into the oöcyst.--Auth. summ.

FIG. 2. Sample bibliography card. A, first parasite genus (*222* = *Eimeria*); B, second parasite genus (*224* = *Tyzzeria*); D, host (*36* = geese); E, first author (*–12* = L); G, first subject (*–01* = taxonomy); H, second subject (*x 02* = epidemiology).

for 99 numbers is used for the host. An additive code based on five holes is used for the most important subject of the paper; another group of five holes is used for a second subject. An additive code based on five holes is used for the first letter of the first author's name. Finally, three single holes are punched separately in case the paper discusses more than two parasite genera, more than one host, or more than two subjects, respectively.

This leaves a group of seven holes which are available for other information one might wish to code, such as species of certain parasite genera or types of chemotherapuetic agents. With an additive code, this would allow for 120 numbers.

An example of a completed card is shown in Fig. 2. As a matter of convenience, the date of the paper is written on the card in the upper left just below the printed numbers. The volume of *Biological Abstracts* from which the abstract was clipped is written in the upper right corner.

It has been found that if the corner holes are to be punched out, it is best to clip off the projecting tags at the top or bottom. Otherwise these may get into the holes of other cards during handling and prevent the cards from stacking smoothly.

In the parasite code, only numbers 100–999 are used for animal parasites. Numbers 1–99 are reserved for bacteria, rickettsiae, viruses, spirochetes and fungi, and are not included here. If desired, these numbers could be used to expand the code of a particular group of parasites. For example, in the present only 100 numbers are used for the TURBELLARIA and TREMATODA; this number could be doubled if one wished.

In preparing the parasite code, the classification of the PROTOZOA was based on Kudo (1946), that of the TREMATODA on Dawes (1946), that of the CESTODA on Wardle and McLeod (1952), that of the NEMATODA on Chitwood (1950); and that of all ARTHROPODA except the ACARINA on Ross (1948); for the ACARINA, Baker and Wharton (1952) was used. Since this last book appeared after the code had been worked out, it was necessary to modify the code somewhat in order to make it conform to the new classification. As a result, there is a little irregularity in the number sequence, but this is of no great consequence .

It will be noted in the parasite code that, in general, numbers which end in 0 have been used for groups, while numbers which end in digits have been used for individual genera. In some cases, because of the large number of families and other groups, it was necessary to assign numbers ending in digits to some of them.

Vacant numbers have been left wherever possible in order to permit future expansion of the code. When this has been done, an attempt has been made to use numbers ending in 1, 2, 4 or 7 for the more important genera, since this decreases the number of holes which must be punched out.

Separate numbers have not been assigned to many parasite genera; as a matter of fact, many families and even higher groups have not received numbers. For example, only 20 families of digenetic trematodes have been given them, yet Dawes (1946) included 48 in his book, and stated that there are over 60 families plus many genera which have not so far been allocated to families. As another example, the protozoan subclasses PHYTOMASTIGINA, ACNIDOSPORIDIA and CNIDOSPORIDIA have not been divided beyond orders, even though further subdivisions might be useful. However, it would be impossible within the physical limits imposed by the number of holes available on the cards to assign separate numbers to every genus or even to every family. To do so would actually make the code less convenient to use and would partially defeat its purposes. This code is not intended to be complete, nor are the taxonomic categories on which it is based to be definitive ones. The code is intended simply to be practical and convenient. It could still be used even if some of the genera were shifted around; this has already been demonstrated by the changes made after Baker and Wharton's (1952) classification of ACARINA was published.

It is not necessary to file the cards in any particular order. As a matter of fact, as the cards are sorted for different types of information, they will become more or less shuffled. It may, however, be found convenient to keep them sorted by phylum of parasite.

PARASITE CODE

(12 holes; decimal code; 999 numbers)

Phylum PROTOZOA	100-299
Class MASTIGOPHORA	100-169
Subclass PHYTOMASTIGINA	101-109
Order CHRYSOMONADINA	102
Order CRYPTOMONADINA	103
Order PHYTOMONADINA	104
Order EUGLENOIDINA	105
Order DINOFLAGELLATA	106
Subclass ZOOMASTIGINA	110-169
Order RHIZOMASTIGINA	111-112
Histomonas	112
Order PROTOMONADINA	120-139
Family TRYPANOSOMATIDAE	120-129
Crithidia	122
Herpetomonas	124
Leishmania	127
Leptomonas	123
Phytomonas	125
Trypanosoma	121
Family BODONIDAE	130-139
Bodo	131
Proteromonas (Prowazekella)	133
Retortamonas	132
Trypanophis	134
Order POLYMASTIGINA	140-159
Suborder MONOMONADINA	140-149
Chilomastix	144
Cochlosoma	147
Enteromonas	146
Hexamastix	148
Monocercomonoides	142
Tetramitus	145
Trichomonas	141
Suborder DIPLOMONADINA	150-156
Giardia	152
Hexamita	151
Suborder POLYMONADINA	157-159
Order HYPERMASTIGINA	160-169
Barbulanympha	162
Trichonympha	161
Class SARCODINA	170-199
Subclass RHIZOPODA	170-191
Order PROTEOMYXA	171
Order MYCETOZOA	172
Order AMOEBINA	173-189
Family DIMASTIGAMOEBIDAE	174
Family AMOEBIDAE	175-178
Amoeba	176
Hartmannella	178
Pelomyxa	177
Family ENDAMOEBIDAE	180-189
Dientamoeba	187
Endamoeba	181
Endolimax	185
Entamoeba	182
Iodamoeba	184
Family PARAMOEBIDAE	179
Order TESTACEA	190
Order FORAMINIFERA	191
Subclass ACTINOPODA	192-193
Order HELIOZOA	192
Order RADIOLARIA	193
Class SPOROZOA	200-279
Subclass TELOSPORIDIA	201-274
Order GREGARINIDA	201-209
Gregarina	204
Monocystis	202
Order COCCIDIA	210-249
Suborder EIMERIDIA	210-239
Family SELENOCOCCIDIIDAE	211
Family AGGREGATIDAE	212-216
Family DOBELLIIDAE	217-219
Family EIMERIIDAE	220-239
Barrouxia	234
Caryospora	228
Cryptosporidium	229
Cyclospora	225
Dorisiella	226
Echinospora	235
Eimeria	222
Globidium	236
Isospora	221
Jarrina	223
Lankesterella	232
Pfeifferinella	231
Schellackia	233
Tyzzeria	224
Wenyonella	227
Suborder ADELEIDEA	240-249
Family ADELEIDAE	240
Family HAEMOGREGARINIDAE	241-249
Haemogregarina	242
Hepatozoon	244
Karyolysus	247
Order HAEMOSPORIDIA	250-274
Family PLASMODIIDAE	251-252
Plasmodium	252
Family HAEMOPROTEIDAE	253-259
Haemoproteus	254
Leucocytozoon	257
Family BABESIIDAE	260-269
Babesia	261
Dactylosoma	265
Echinozoon	263
Nuttallia	262
Theileria	264
Toxoplasma	267
INCERTAE SEDIS	270-274
Aegyptianella	273
Anaplasma	271
Eperythrozoon	272
Sarcocystis	274
Subclass ACNIDOSPORIDIA	275
Subclass CNIDOSPORIDIA	276-279
Order MYXOSPORIDIA	276
Order ACTINOMYXIDIA	277
Order MICROSPORIDIA	278
Order HELICOSPORIDIA	279
Class CILIATA	280-298
Subclass PROTOCILIATA	281
Subclass EUCILIATA	282-298
Order HOLOTRICHA	282-289
Colpidium	286
Colpoda	285
Ichthyophthirius	283
Paramecium	284
Tetrahymena	287
Equid holotrichs	288
Ruminant holotrichs	289
Order SPIROTRICHA	290-296
Suborder HETEROTRICHA	290-292
Balantidium	291
Nyctotherus	292
Suborder OLIGOTRICHA	293-295

Family OPHRYOSCOLECIDAE 294
Family CYCLOPOSTHIIDAE 295
Suborder HYPOTRICHA 296
Order CHONOTRICHA 297
Order PERITRICHA 298
Class SUCTORIA 299

Phylum MESOZOA 960-969

Phylum PLATHELMINTHES 300-499
Class TURBELLARIA 301
Class TREMATODA 300-399
Order MONOGENEA 302
Order ASPIDOGASTREA 303
Order DIGENEA 305-399
Family PLAGIORCHIIDAE 310-314
Plagiorchis 311
Prosthogonimus 312
Schistogonimus 314
Family DICROCOELIIDAE 315-317
Dicrocoelium 315
Eurytrema 316
Platynosum 317
Family MICROPHALLIDAE 319
Levinseniella 319
Family OPISTHORCHIIDAE 320-326
Amphimerus 325
Metorchis 321
Opisthorchis (= Clonorchis) 324
Parametorchis 322
Pseudamphistomum 323
Family HETEROPHYIDAE 330-338
Adleriella 338
Apophallus 334
Cryptocotyle 333
Euryhelmis 335
Heterophyes 331
Metagonimus 332
Phagicola 336
Pygidiopsis 337
Family PHILOPHTHALMIDAE 340-341
Philophthalmus 341
Family PSILOSTOMATIDAE 342-343
Ribeiroia 342
Sphaeridiotrema 343
Family EUCOTYLIDAE 344
Tamerlanea 344
Family TROGLOTREMATIDAE 345-348
Collyriclum 345
Paragonimus 347
Renicola 346
Troglotrema 348
Family CYCLOCOELIDAE 327-329
Cyclocoelum 327
Hyptiasmus 328
Typhlocoelum 329
Family NOTOCOTYLIDAE 306-308
Catatropis 306
Notocotylus 307
Paramonostomum 308
Family BRACHYLAIMIDAE 350-353
Brachylaima 351
Hasstilesia 353
Leucochloridium 352
Family ECHINOSTOMATIDAE 354-358
Echinochasmus 357
Echinoparyphium 354
Echinostoma 355
Euparyphium 356
Hypoderaeum 358
Family FASCIOLIDAE 360-364
Fasciola 361
Fascioloides 362
Fasciolopsis 364
Family PARAMPHISTOMATIDAE 370-378
Cladorchis 375
Gastrodiscus 371
Gastrothylax 373
Homalogaster 372
Paramphistomum 377
Watsonius 378
Zygocotyle 374
Family CYATHOCOTYLIDAE 367-369
Cyathocotyle 367
Mesostephanus 368
Pharyngostomum 369
Family STRIGEIDAE 380-384
Apatemon 381
Cotylurus 382
Parastrigea 383
Strigea 384
Family DIPLOSTOMATIDAE 386-387
Alaria 387
Diplostomum 386
Family CLINOSTOMATIDAE 389
Clinostomum 389
Family SCHISTOSOMATIDAE 390-398
Bilharziella 391
Dendritobilharzia 395
Gigantobilharzia 392
Ornithobilharzia 396
Pseudobilharzia 393
Schistosoma 397
Schistosomatium 394
Trichobilharzia 398
CERCARIAE OF UNKNOWN ADULTS 399
Class CESTODARIA 490-499
Class CESTODA 400-489
Order CYCLOPHYLLIDEA 410-479
Family MESOCESTOIDIDAE 411-412
Mesocestoides 412
Family ANOPLOCEPHALIDAE 420-439
Andrya 421
Anoplocephala 427
Aporina 428
Avitellina 435
Bertiella 425
Cittotaenia 426
Helictrometra 434
Moniezia 424
Moneococestus 422
Oochoristica 433
Paranoplocephala 429
Stilesia 432
Thysanosoma 431
Family TAENIIDAE 440-449
Echinococcus 441
Hydatigera 443
Multiceps 444
Taenia 447
Taeniarhynchus 442
Family DAVAINEIDAE 450-459
Davainea 451
Raillietina 452
Family HYMENOLEPIDIDAE 460-469
Diorchis 462
Fimbriaria 464
Hymenolepis 461
Family DILEPIDIDAE 470-479
Amoebotaenia 471
Choanotaenia 472
Dilepis 473
Diplopylidium 475
Dipylidium 474

Joyeuxiella 476
Metroliasthes 477
Order PSEUDOPHYLLIDEA 480-489
Cordicephalus 483
Dibothriocephalus 487
Diphyllobothrium 482
Ligula 481
Schistocephalus 488
Spirometra 484
Triaenophorus 489

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Phylum NEMATODA 500-689
Class PHASMIDIA 500-649
Order RHABDITIDA 501-509
Suborder RHABDITINA 502-509
Superfamily RHABDITOIDEA 503-509
Neoaplectana 506
Rhabdias 505
Rhabditis 504
Strongyloides 507
Suborder STRONGYLINA 510-569
Superfamily STRONGYLOIDEA 510-529
Agriostomum 527
Ancylostoma 514
Bunostomum 517
Chabertia 518
Codiostomum 523
Gaigeria 519
Globocephalus 525
Gyalocephalus 526
Necator 515
Oesophagostomum 521
Stephanurus 522
Strongylus 512
"small horse strongyles" 513
Syngamus 524
Ternidens 528
Uncinaria 516
Superfamily TRICHOSTRONGYLOIDEA 530-549
Amidostomum 544
Cooperia 547
Graphidium 536
Haemonchus 531
Hyostrongylus 537
Longistriata 543
Mecistocirrus 535
Molineus 533
Nematodirus 541
Nippostrongylus 542
Obeliscoides 538
Ollulanus 539
Ornithostrongylus 545
Ostertagia 532
Oswaldocruzia 546
Trichostrongylus 534
Superfamily METASTRONGYLOIDEA 550-569
Aelurostrongylus 563
Angiostrongylus 558
Crenosoma 562
Dictyocaulus 551
Filaroides 561
Metastrongylus 552
Metathelazia 564
Muellerius 554
Protostrongylus 557
Skrjabingylus 565
Suborder ASCARIDINA 570-599
Superfamily OXYUROIDEA 570-579
Aspiculuris 574
Enterobius 571
Leidynema 573
Oxyuris 572
Passalurus 577
Skrjabinema 578
Syphacia 575
Superfamily ASCARIDOIDEA 580-589
Anisakis 586
Ascaridia 584
Ascaris 581
Contracaecum 585
Cosmocercoides 589
Heterakis 591
Neoascaris 583
Parascaris 582
Porrocaecum 595
Probstmayria 594
Subulura 592
Toxascaris 588
Toxocara 587
Order SPIRURIDA 600-649
Suborder CAMALLANINA 601-609
Superfamily DRACUNCULOIDEA 602-609
Dracunculus 604
Philometra 607
Suborder SPIRURINA 610-649
Superfamily SPIRUROIDEA 610-629
Acuaria 614
Ascarops 612
Cheilospirura 615
Dispharynx 616
Draschia 618
Gnathostoma 611
Gongylonema 621
Habronema 617
Hartertia 623
Oxyspirura 622
Physaloptera 624
Physocephalus 613
Rictularia 619
Simondsia 625
Spirocerca 626
Tetrameres 628
Thelazia 627
Superfamily FILARIOIDEA 630-649
Dipetalonema 639
Diplotriaena 648
Dirofilaria 637
Elaeophora 644
Filaria 636
Litomosoides 647
Loa 633
Mansonella 635
Neurofilaria 646
Onchocerca 634
Parafilaria 638
Setaria 641
Stephanofilaria 642
Suifilaria 643
Vagrifilaria 645
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Class APHASMIDIA 650-689
Order ENOPLIDA 650-689
Suborder DORYLAIMINA 650-659
Superfamily MERMITHOIDEA 651
Superfamily TRICHUROIDEA 652-659
Capillaria 654
Trichinella 652
Trichosomoides 653
Trichurus 657
Suborder DIOCTOPHYMATINA 660-669
Dioctophyma 661
Eustrongylus 662

Hystrichis 664
Soboliphyme 667

Phylum ACANTHOCEPHALA 910-929
Centrorhynchus 913
Corynosoma 911
Echinorhynchus 912
Filicollis 914
Leiperacanthus 915
Leptorhynchoides 916
Macracanthorhynchus 917
Mediorhynchus 918
Moniliformis 921
Neoechinorhynchus 922
Oncicola 924
Plagiorhynchus 925
Polymorphus 927

Phylum RHYNCHOCOELA (NEMERTINA) 950-959

Phylum ANNELIDA 930-939

Phylum MOLLUSCA 940-949

Phylum ARTHROPODA 700-899, 970-989
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Subclass COPEPODA 970-979
Subclass CIRRIPEDIA 980-989
Class MYRIAPODA 701
Class INSECTA 700-809
Order ORTHOPTERA 704
Order MALLOPHAGA 710-719
Bovicola 711
Columbicola 712
Cuclutogaster 713
Eomenacanthus 714
Felicola 718
Goniocotes 716
Heterodoxus 719
Lipeurus 715
Trichodectes 717
Order ANOPLURA 720-729
Haematopinus 721
Hoplopleura 722
Linognathus 723
Pediculus 724
Phthirius 725
Polyplax 728
Solenopotes 727
Order HEMIPTERA 730-739
Cimex 731
Panstrongylus 732
Rhodnius 734
Triatoma 733
Order HYMENOPTERA 705
Order COLEOPTERA 706
Order DIPTERA 740-799
Suborder NEMATOCERA 740-759
Family CERATOPOGONIDAE 741-743
Culicoides 742
Family SIMULIIDAE 744-746
Eusimulium 745
Simulium 744
Family PSYCHODIDAE 747-749
Phlebotomus 747
Family CULICIDAE 750-759
Aedes 751
Anopheles 757
Culex 752
Haemagogus 753
Mansonia 755
Psorophora 754
Suborder BRACHYCERA 760-769
Family TABANIDAE 761-766
Chrysops 764
Tabanus 762
Family RHAGIONIDAE 767-769
Rhagio 767
Suborder ATHERICERA 770-799
Family MUSCIDAE 770-776
Glossina 776
Haematobia 772
Lyperosia 773
Musca 774
Muscina 775
Stomoxys 771
Family CALLIPHORIDAE 780-788
Auchmeromyia 781
Calliphora 784
Callitroga 782
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Psoroptes	892
Sarcoptes	894
Group ORIBATEI	870-879
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SUBJECT CODE

(5 holes; additive code; 30 numbers)

Biometry	-06*	Growth and Regeneration	-10
Bionomics of Ova and Larvae	-13	History and Biography	-03
Chemotherapy and Prophylaxis	x12*	Host-Parasite Relations	x03
Control	x01	Immunology	x05
Cultivation	x11	Life Cycle	-08
Diagnosis	-14	Metabolism	x07
Ecology and Biology	-07	Morphology and Cytology	-02
Effects of Chemicals	x13	Nutrition	x08
Effects of Physical Agents	x14	Pathogenesis	x04
Encystment and Excystment	-11	Physiology	x06
Epidemiology; Epizootiology; Transmission; Geographic Distribution; Case Reports	x02	Reproduction	-09
		Respiration	x10
		Taxonomy	-01
Evolution	-05	Technics	x00
Excretion	x09	Other	-12
Genetics	-04		

* x = 5th hole punched out; - = 5th hole not punched out.

HOST CODE

(8 holes; decimal code; 99 numbers)

General	10	Ducks	35
Plants	01-09	Geese	36
Phylum PROTOZOA	11	Order FALCONIFORMES	55
Phylum PLATHELMINTHES	12	Order GALLIFORMES	41
Class TREMATODA	13	Chicken	42
Class CESTODA	14	Guinea fowl	43
Phylum NEMATODA	15	Peafowl	44
Phylum ANNELIDA	16	Pheasant	45
Phylum MOLLUSCA	17	Quail	46
Phylum ARTHROPODA	18	Turkey	47
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Class ARACHNIDA	22	Doves	52
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FIRST AUTHOR CODE

(5 holes; additive code; 30 numbers)

A	-01	J	-10	R	x05
B	-02	K	-12	S	x06
C	-03	L	-12	T	x07
D	-04	Mc	-13	U	x08
E	-05	M	-14	V	x09
F	-06	N	x01	W	x10
G	-07	O	x02	X	x11
H	-08	P	x03	Y	x12
I	-09	Q	x04	Z	x13

SUMMARY

A coding system is described for the use of 3 by 5 punched cards in filing abstracts and references to the parasitological literature. Codes are given for punching information on parasite taxonomic group and genus, subject, host and author around the edges of the cards. The cards can then be searched rapidly with a sorting needle for different types of information.

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OBSERVATIONS ON TOXOPLASMOSIS IN DOGS

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There is no question that under some circumstances *Toxoplasma gondii* is the occasional cause of a severe disease of dogs. Since Mello described the first spontaneous case of canine toxoplasmosis in 1910, over 50 cases of the infection in dogs have been reported from all parts of the world (for review, see Habegger, 1953). Some of these reports have described a fatal disease, manifested by such symptoms as severe respiratory distress, diarrhea, convulsions, and partial paralysis. On the other hand, some of the reports merely record the finding of toxoplasmas in histological sections of dog tissues, indicating that *Toxoplasma* may exist in dogs without producing symptoms.

There is also serological evidence that dogs are frequently found infected in nature. For example, Miller and Feldman (1953) have reported the finding of anti-*Toxoplasma* antibodies in 59 percent of 51 dogs investigated. This naturally has led to the conjecture expressed by various authors (Chamberlain *et al.*, 1953; Fankhauser, 1951; Cole et al., 1953; Westphal and Finke, 1950) that the dog may serve as a reservoir of human infection. Some surveys have indicated a correlation between antibodies in dogs and in their owners and handlers (Otten, Westphal, and Kajahn, 1951) although the serological procedures used give results different from those reported by other investigators.

The widespread occurrence of anti-*Toxoplasma* antibodies among humans and dogs makes it extremely difficult to decide in any particular case that the dog infection manifested by such antibodies was related even in time to human infection. Moreover, the histories of mothers of cases of congenital toxoplasmosis sometimes have contained denials of any even casual contacts with dogs. For example, the mother of one congenital case that we have studied serologically claims that dogs are not allowed in the large apartment development in which she lives and that she hardly ever saw dogs, let alone came in close contact with them, during the time of her pregnancy. The question, therefore, of the epidemiological importance of dogs in regard to human toxoplasmosis has remained obscure.

In order to judge the role of the dog in the spread of *Toxoplasma*, information in regard to the manner by which the parasite might be transmitted from the dog to other animals and man must be accumulated. Some statements can be found on this point in the literature. For example, the pneumonitis described in the acute disease and the finding of toxoplasmas in the alveoli of the lungs (MacHattie, 1938; Olafson and Monlux, 1942) suggest that the organism might be present in sputum from the animals. Also, the parasite has been found in the urine and feces of animals with the acute disease (see Jacobs, 1953 for a review of these observations). Consequently, even though toxoplasmas in the only stage in which we know them

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are not resistant to the external environment, the possibilities do exist that on close contact viable organisms might be transmitted from one animal to another. Indeed the report of Olafson and Monlux contains a record of apparently direct transmission from one sick puppy to its cage mates.

All of these findings have been made with acutely sick animals. It appeared worthwhile to study the infection in dogs to see under what circumstances acute disease is produced, and whether or not those animals not manifestly ill from the infection can nevertheless serve as reservoirs for its spread. Our interests were mainly in ascertaining the parasitemia in infected dogs, and the infectivity of feces and urine of animals given parenteral and oral infections. Some observations were made on the relation of age of the animals to the outcome of experimental infection and on the serological response of the animals.

MATERIALS AND METHODS

Twenty-three dogs have been used in these studies. All except two were of known age. Two litters, one of 5 and the other of 6 puppies, were received from local sources soon after weaning. The other animals were procured from a dealer or represented young animals turned over to us by their owners. Of these dogs, four were fed *Toxoplasma*-infected tissues. The others were all given parenteral inoculation with virulent *Toxoplasma* of the RH strain. Most of the parental infections were intravenous, but some were given intradermally with the idea that the development of local lesions at the site of inoculation would allow assurance that the infection had been established. Differences between the intradermal and intravenous routes as regards ultimate outcome of infection and parasitemia have been found to be only slight in previous work on rabbits (Jacobs and Jones, 1950).

Inocula containing calculated numbers of parasites were prepared from the peritoneal exudates of intraperitoneally infected mice. The organisms were counted in Neubauer-Levy counting chambers and appropriate dilutions in 0.85 percent saline containing 10 percent normal serum were made to give suspensions of the desired numbers of parasites. Intravenous inoculations were done in the vena saphena or the jugular; blood for parasitemia studies was generally taken from the saphena. Blood was tested for the presence of parasites by inoculating it, undiluted or in dilutions of up to 1 : 1000 in saline, into mice. Fecal specimens were collected from the cages of some of the animals on a number of occasions early and late in the course of infection. These were emulsified in saline or water and force-fed to mice by means of a blunt needle. Sometimes feces were also mixed in the drinking water provided to mice. In other cases the feces were saved in a dry or slightly moist state and after storage for various intervals were force-fed to mice. By the use of metabolism cages, it was possible to collect urine from some of the dogs. This was centrifuged, resuspended in saline, and inoculated into mice. When dogs died or were sacrificed, suspensions of their organs were macerated in saline and inoculated into mice to determine the presence of the parasites. All such inoculations into mice were done intraperitoneally.

The serum of the animals was tested by the dye test of Sabin and Feldman (1948). The procedures used were those outlined by Jacobs and Cook (1954).

RESULTS

Table 1 presents a summary of experimental attempts at producing toxoplasmosis in dogs by the feeding of infected tissues. The results of parasitemia tests on these animals were uniformly negative. The only evidence of the possibility that some invasion by *Toxoplasma* may have taken place is the relatively high dye test titer found in dog 8 approximately 3 weeks after infection. Dog 6 responded also with a low and transient antibody titer. Urine collected from dog 5 on days 9, 10, 12, 15, 18, 23, and 24 and from dog 6 on days 1, 2, 3, 4, 7, 9, 10, 14, and 15 did not produce toxoplasmosis when inoculated into mice. Feces of dog 8, collected on days 4, 9, 10, 16, and 22 and of dog 11 collected on days 1, 2, 4, 7, 8, and 9 similarly gave negative results when fed to mice. Dog 6 was sacrificed on day 21 and was found negative on subinoculation of its tissues. Dog 8 was sacrificed at a much later date, over 2 years after infection, and was negative on subinoculation. Dogs 5 and 11 were not sacrificed but were used later for parenteral infection.

TABLE 1.—*Results of feeding infected tissues to dogs*

Dog no.	Age	Tissues fed	Days tested for parasitemia	Results	Serological tests
5	6.5 weeks	Rabbit liver-CH	3, 5, 7, 10, 12, 14, 18, 19, 21, 24	Negative	Negative
6	6.5 weeks	Rabbit liver-RH	3, 5, 7, 10, 12, 14, 18, 19, 21	Negative	Negative before feeding Day 11—1 : 16 to 1 : 64 Day 18—1 : 1
8	3 months	Rabbit liver-RH	6, 8, 13, 15, 17, 20	Negative	Negative before feeding Day 8—1 : 1 Day 13—Negative Day 20—1 : 256 Day 42—1 : 256 Day 120—1 : 256 Day 180—1 : 32
11	Mature	Rat brains and livers-RH	Not done		Negative

Table 2 summarizes the results of parenteral infection of dogs with virulent toxoplasmas. It is to be noted that the only group of animals in which deaths resulted from the inoculations was the very young group, consisting of puppies infected at 5 weeks or 6.5 weeks of age. Similarly, the only animals in which parasitemia was demonstrated were these young puppies. While the two mature dogs of uncertain age received intradermal inoculations, all the other age groups contained some animals which were inoculated intravenously with comparable numbers of parasites. Hence, the only factor to which the difference in response appears to be attributable is age.

TABLE 2.—*Summary of dogs infected parenterally with RH toxoplasmas*

Age	Total no. of dogs	Parasitemia No. pos./no. tested	Number of deaths
45 days or less	8	7/8*	4
65 days	1	0/1*	0
4 months	6	0/3*	0
7 to 9 months	2	0/2*	0
Mature (age unknown)	2	0/2*	0

* Parasitemia was tested about 3 times per week for first 3 weeks after infection.

A further indication of the effect of age differences, even relatively small ones, can be found in Table 3. Here, of two litter mates, one infected at 5 weeks with

TABLE 3.—*Comparison of litter mate infections*

Litter No. 1

Dog No.	Age at infection	How infected	Outcome
15	5 weeks	100,000 IV	Death—8 days
21	9.5 weeks	2,000,000 IV	Survived
17	3.5 months	10,000 IV	Survived
18	6.5 weeks	10,000 IV	Survived
19*	3.5 months	10,000 IV	Sacrificed (no symptoms)
20*	3.5 months	10,000 IV	Survived

Litter No. 2

Dog No.	Age at infection	How infected	Concomitant infection	Outcome
22	5 weeks	10,000 IV		Survived
23	5 weeks	100,000 IV		Death—7 days
24	6.5 weeks	10,000 IV	80 hookworm larvae (day–9)	Survived
25	6.5 weeks	10,000 IV	200 hookworm larvae (day–3)	Death—7 days
26	6.5 weeks	100,000 IV		Death—6 days

* Dogs 19 and 20 had been kept in same cage with dog 15 during its acute infection. They remained healthy and negative serologically.

100,000 parasites intravenously succumbed to the infection. The second, given 2 million parasites by the same route one month later, survived the infection and remained asymptomatic.

Another factor which may be involved in susceptibility to toxoplasmosis is suggested in Table 3, comparing the pups of litter No. 2. Here, of two puppies 6.5 weeks of age, one given a large dose of hookworm larvae on day 3 of the infection succumbed to toxoplasmosis. Another puppy, given a smaller dose of hookworms 9 days prior to infection, suffered no disease. An intravenous inoculation of 10,000 RH parasites also failed to produce disease in another pup of this litter infected at 5 weeks of age. Concomitant infections may, therefore, play a role in the outcome of canine toxoplasmosis.

Those puppies which succumbed to toxoplasmosis showed parasitemia as early as day 3 after infection. On day 4, 0.5 ml of blood from one of these animals killed mice in 7 days. Dilutions of 1 : 10, 1 : 100, and 1 : 1000 of whole blood from one sick puppy on day 7 killed mice in 7, 8, and 9 days respectively. Parasitemia was studied in four puppies which survived RH infections (Table 4). One puppy, number 7, infected intradermally with 5000 RH parasites at 6.5 weeks of age showed no parasitemia. Dog 22 was infected at 5 weeks, and the other two at 6.5 weeks of age, all

TABLE 4.—*Parasitemia in puppies surviving RH* Toxoplasma *infection*

Dog no.	How infected	Days tested	Days positive	Dilutions positive	Results	
					No. mice dead*	Survival days of inoculated mice
7	5,000 ID	3, 4, 7, 9, 11, 15, 16, 18	None			
18	10,000 IV	3, 7, 10, 17	7	WB	2	10, 11
22	10,000 IV	4, 10, 12, 14, 18, 21	4	WB	1	10
			10	WB	2	8
			12	WB	2	8
			12	1 : 10	2	8
			14	WB†	2	10
24	10,000 IV	3, 7, 10	7	WB	2	8, 9
			7	1 : 10	2	9, 11
			7	1 : 100	2	9
			10	WB†	1	14

* 2 mice were inoculated with each blood sample.
† Higher dilutions were negative. WB = whole blood.

intravenously with 10,000 RH parasites. The earliest death of mice inoculated with whole blood from these animals occurred in 8 days, indicating a lower parasitemia than in the sick animals. The parasitemia in surviving animals was transient, as evidenced by failure to produce infections in mice inoculated with blood drawn on days 10 and 17 from dog 18 and on days 18 and 21 from dog 22, and by the increased survival time of mice inoculated on days 10 and 14 from dogs 24 and 22 respectively.

The disappearance of parasitemia appeared to be coincidental with the elaboration of antibodies revealed by the dye test. Specifically, at 2 weeks dog 7 had a titer of 1:256; dog 18, 1:4096; and dog 22, 1:16,000. As can be seen from Table 5, all dogs tested at 2 weeks after inoculation showed antibodies at a titer of 1:256 or higher.

Table 5 also shows that, in general, dye test antibodies returned rather early to relatively low levels. Titers of 1:64 and even 1:16 were found 3 and 4 months

TABLE 5.—*Serological response of dogs surviving parenteral infection*

Weeks after infections	Number of dogs showing titers of								Total tested
	0	1:16	1:64	1:256	1:1,024	1:4,096	1:16,000	1:65,000	
1	1	4	2	1	1				9
2				3		2	2	1	8
4				1	2	3	2	2	10
6					4				4
8					4	1	1	1	7
12			2	3					5
16		1	2	1					4
26			1	2	2				5
39		1		2					3
52			1	1					2

after infection. In only 2 of 5 animals tested at 6 months was the titer found to be 1:1024. Some fluctuations in titer of individual dogs occurred in tests done 3 to 12 months after infection, but these were no greater than four-fold, *i.e.* a difference of one dilution tube. Dogs infected intradermally showed just as high antibody titers as those which received intravenous inoculations. Titers of 1:16 to 1:128 did, however, persist for over 2 years after infection.

Thirteen of the animals which received parenteral infections were examined for the presence of parasites in their various organs at the time they were sacrificed. Portions of the brain, lung, liver, spleen, and sometimes the heart were inoculated as 10 percent suspensions (weight/volume) into mice. The results are shown in Table 6. The only positive results obtained with the tissues of animals sacrificed one month or longer after infection were with dogs which were infected when very

TABLE 6.—*Demonstration of parasites in dogs surviving parenteral RH infection*

Age	No. of dogs	Time of sacrifice	No. dogs		Organs positive	Survival days of inoculated mice
			Positive	Negative		
45 days or less	4	1 mo., 2.5 mos.	0	2		
		9 mos., 10 mos.	2	0	Brain	8, 9
65 days	2	10 mos., or more	1	1	Liver	9
4 mos.	5	1 week	1	0	Lung	6, 7
					Liver	7, 8
					Spleen	5, 7
		2 weeks	1	0	Lung	9
					Liver	9
					Spleen	9
		9 mos. or more	0	3		
7 to 9 mos.	2	3 mos., 10 mos.	0	2		

young, 65 days in age or less. Here, however, such findings do not show any particular pattern. For example, the brains of puppies infected at 45 days of age and sacrificed at 1 and 2.5 months were not found positive, while toxoplasmas were isolated from the brains of two others killed at 9 and 10 months. In one dog infected at 65 days, and killed at 10 months the liver was found positive but not the brain. It appears, therefore, that the isolation of *Toxoplasma* from the tissues of dogs is fortuitous, and that it may not be accomplished even in animals with latent infections.

The two dogs sacrificed 1 and 2 weeks after inoculation were selected for this study because they were old enough so that they could be expected to survive the infection. The purpose was to determine the presence and distribution of parasites in asymptomatic animals. Both dogs received 10,000 RH parasites intravenously. It is to be noted that at one week the parasites were abundantly distributed in the lung, liver, and spleen of the dog, as evidenced by the rapid death of mice inoculated with the suspensions of these organs. The data on the second dog, sacrificed at 2 weeks, indicate that a diminution in the numbers of parasites in these organs was already occurring. Mice inoculated with comparable tissue suspensions died in 9 days rather than in 5 to 8 days, as was the case with the tissues of the dog killed at 1 week. Parasitemia studies were not done on these two animals. However, it is believed that the results on infectivity of the tissues of these two dogs can be correlated with the observed diminution of parasitemia in puppies at 10 to 14 days.

Urine from these two animals was also found infective to mice. Here again the same relationship was observed. While the urine from the dog sacrificed at 1 week produced toxoplasmosis fatal in 9 days to all of 3 mice inoculated with it, a comparable sample from the dog sacrificed at 2 weeks killed only 1 of 3 mice so inoculated.

Puppies which died of acute toxoplasmosis showed parasites abundantly distributed in the brain, lung, liver, spleen, heart, and kidney. The lung and liver appeared to be most heavily parasitized; suspensions prepared from these organs killed mice in 5 and 6 days, while mice inoculated with the other organ suspensions survived 1 to 3 days longer. Urine from these puppies was also positive for toxoplasmas; 1.0 ml of urine centrifuged and resuspended in saline killed mice in 8 to 9 days. It can be remarked here that the lung and liver were the only organs of the puppies which showed significant gross pathology. The lungs were markedly congested and hemorrhagic and the liver revealed numerous yellowish white areas of necrosis. No macroscopic intestinal lesions were seen in any of the dogs examined.

Feces were collected from 8 of the dogs which survived parenteral inoculations of toxoplasmas, and urine was collected from 2 of these animals. All attempts to produce toxoplasmosis in mice by the feeding or inoculation of these excreta gave negative results.

DISCUSSION

Chamberlain *et al.* (1953) were able to produce acute toxoplasmosis in 5 bitches 1.5 to 2 years of age by the intravenous inoculation of very large numbers of *Toxoplasma*. For example, their animals were inoculated with from 1 ml to 2.25 ml of peritoneal exudate from infected mice. One ml of such exudate usually contains from 30 million to 200 million parasites. Despite these extremely large inocula, and despite the fact that they were supplemented by additional injections subcutaneously or intraperitoneally, or by oral administration of large numbers of toxoplas-

mas, only 2 deaths occurred among these animals, and one bitch remained entirely asymptomatic. This was true even though all the bitches were pregnant when inoculated, and all gave birth to pups with toxoplasmosis.

It is not surprising, therefore, that the use of smaller inocula in our studies failed to result in the development of acute toxoplasmosis except in very young animals. Other reports in the literature coincide with these observations. For example, Yakimoff and Kohl-Yakimoff (1911), Nicolle and Conor (1913) and Boez (1921) failed to produce disease in dogs by the inoculation of *Toxoplasma* by various routes. Laveran and Marullaz (1913) and Mesnil and Sarrailhé (1913) produced disease in young puppies by intravenous inoculation of the parasites. Westphal and Finke (1950) could not demonstrate toxoplasmas in mature dogs inoculated with virulent organisms.

It is hardly likely that in nature dogs acquire infection by means of exposure to enormous numbers of parasites. It is therefore to be expected, on the basis of our findings, that canine toxoplasmosis is most frequently a subclinical or asymptomatic infection. Since only young animals show acute disease with organisms present in large numbers in the lung or the urine, and since the acute infection even in young animals appears to be of short duration, the chances that dogs serve to spread the infection to humans by any direct means seem to be small. This idea is supported by the fact that *Toxoplasma* is not resistant to the external environment, and that parasites which might be released from the animals in their excreta could not be expected to survive long. In this connection, it may be mentioned that direct transmission was not accomplished in one of our tests in which two 5-week-old uninfected pups were kept in a cage with a litter mate that died of acute toxoplasmosis. The results of our studies with the feces of dogs likewise discount the possibility of this method of transmission. The tests of the feces of dogs fed infected tissues were done with the idea that an alteration to a more resistant form of the parasite might occur on passage through the dog's intestinal tract. No evidence was accumulated to support such an hypothesis. In regard to indirect transmission, e.g. through an arthropod vector, the results reported here do not disprove this possibility. However, in view of the fact that a transient parasitemia was found only in young puppies given intravenous inoculations, it appears that one would have to propose a series of propitious circumstances not only for the infection in the puppies but also for the later transfer of the infection to humans who are exposed only occasionally to mature dogs. While, arthropod transmission merits further study in regard to the epidemiology of toxoplasmosis, there appears to be no reason to consider the dog as the most likely reservoir. The serological correlations between human and canine toxoplasmosis, on which the role of the dog as reservoir has been predicated, may be subject to an alternative explanation. This is that because of their close association dogs and man may acquire the infection from the same source.

Our results on the serological behavior of dogs with *Toxoplasma* infections may allow evaluation of reports relating human infections with illness in pets. For example, Cole *et al.* (1953) state that the pet dog of a woman from whose blood toxoplasmas were isolated in mice showed a titer of 1 : 80 four weeks after an illness. All of our animals which survived parenteral infection with *Toxoplasma* showed higher titers at four weeks; of 10 tested only 1 had a titer as low as 1 : 256. In comparison with the results of Cole *et al.*, also, our success in isolating parasites from

the tissues of animals which survived infection has been markedly less. Possibly differences in strains of parasite may account for these discrepancies. Indeed, the questions raised in the discussion above may be answered on the basis of strain variability. However, we have found strains isolated from asymptomatic animals in nature to be low in virulence. Also, a strain low in virulence for one host has shown similar properties in others (Jacobs and Melton, 1954).

SUMMARY

Only young puppies given relatively large inocula of *Toxoplasma gondii* succumbed to the infection. Parasites were found widely distributed only in puppies which succumbed or in those which were sacrificed at 1 or 2 weeks. Brain, lung, liver, spleen, heart, kidney and urine were found positive. Parasites were rarely demonstrable in animals sacrificed 3 months or longer after infection. Parasitemia was found in the 4 dogs which died, and in 3 additional puppies, none older than 6.5 weeks. In symptomless infections the parasitemia subsided after 10 to 14 days. Antibody response to parenteral inoculation was rapid. Dye test titers of 1 : 16 or 1 : 64 were found at 1 week, 1 : 1024 or higher at 2 weeks. Persistence of high antibody levels varied considerably. Two of 4 dogs fed infected tissues showed transient antibody titers of 1 : 64 to 1 : 256. Feces from the dogs was not found infective, nor was urine except in the case of the young animals that died or were sacrificed at 1 or 2 weeks.

These findings are discussed in relation to the possible role of the dog in the epidemiology of human toxoplasmosis.

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NOTES ON AFRICAN *HAEMAPHYSALIS* TICKS. II. THE GROUND-SQUIRREL PARASITES, *H. CALCARATA* NEUMANN, 1902, AND *H. HOUYI* NUTTALL AND WARBURTON, 1915 (IXODOIDEA, IXODIDAE)*

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The ground-squirrel parasitizing ticks of Africa have been poorly understood because of the paucity of material in most collections, the vague original description of *Haemaphysalis houyi* (= *H. calcarata houyi*) Nuttall and Warburton, 1915, and the uncertainty of limits of variation among subspecies of the superficially similar species, *H. leachii* (Audouin, 1827). Sufficient specimens and data are now at hand to indicate the presence of two readily discernible species of African ground-squirrel parasites, *H. calcarata* Neumann, 1902, and *H. houyi* Nuttall and Warburton, 1915, each with distinctive morphological features, different host species, and separate geographical ranges. These species are distinct from the ubiquitous *H. leachii*, a well known disease vector in Africa, with which *H. houyi* has sometimes been confused for want of any other niche in which to place it. We have reason to believe that *H. leachii* parasitizes ground-squirrels only exceptionally.

The significant potential of ground-squirrels as natural reservoirs of disease-causing organisms and of their tick parasites as vectors of these organisms provides this study with special interest.

HAEMAPHYSALIS CALCARATA Neumann, 1902

Figures 1 to 13, and 40

Haemaphysalis calcarata Neumann, 1902, pp. 113 to 115, description of male and nymph based on 5 males and 1 nymph from "a small squirrel," Ethiopia (without specific locality), Hilgert *legit* (Erlanger collection; specimens said to be at Toulouse); figs. 2 and 3 of male only. Neumann, 1910, pp. 173 and 174, description of supposed female from Roseires (= Roseres), Blue Nile, Anglo-

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Egyptian Sudan, including figs. 11 and 12; this is actually *H. houyi* (see below); also stated that the host of Neumann's type male was *Xerus* (= *Spermosciurus*) sp. Nuttall and Warburton, 1915, pp. 442 to 444, description of adults and nymph based on 4 males, 2 females, and 1 nymph from *Xerus rutilus* subsp. from Bio Caboba (= Biacobaba), Somalia (Somaliland); female description is first pertaining to this sex of *H. calcarata*, also includes note that these females do not correspond with those described by Neumann, 1910, but without details; figs. 373 to 375 of male, original and after Neumann, 1902; fig. 376 of female, original; fig. 377 of nymph, original. Nuttall and Warburton, 1915, pp. 444 to 445, briefly described *houyi* as a variety of *H. calcarata;* also stated that the collecting locality of the type male of *H. calcarata* was Sichi Baz, a place which cannot now be identified. Hoogstraal, 1954a, p. 307, raised *houyi* to full species rank. (Nontaxonomic literature cited in distribution records listed below. Nonoriginal taxonomic literature not listed here.)

MATERIAL EXAMINED: Total 7 males, 6 females

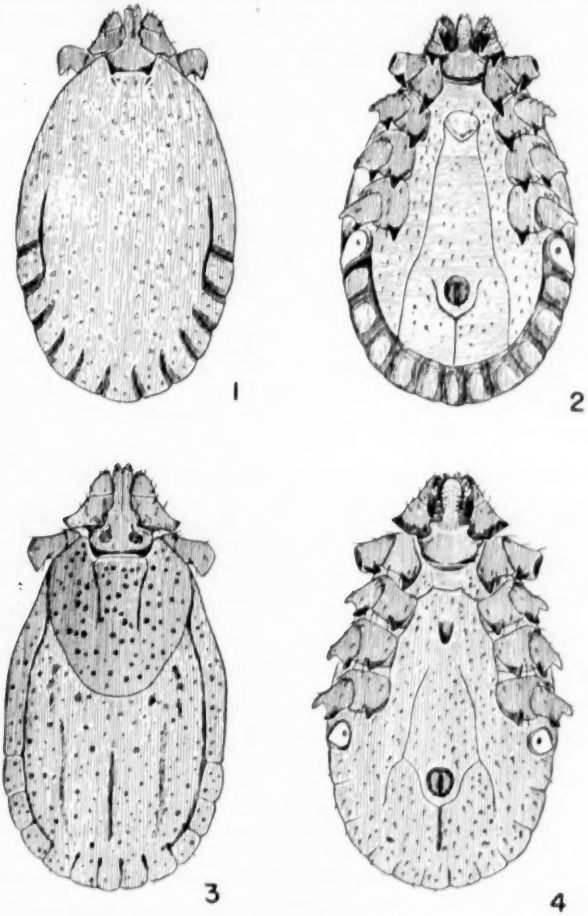
Two males, 1 female from *Xerus rutilus*, Bio Caboba (= Biacobaba), Somalia, April 1901, E. Brumpt, Nuttall lot 1224 (BMNH). [Note: The additional adults and single nymph reported for this lot by Nuttall and Warburton (1915) appear subsequently to have been lost.] A male COTYPE, data as for Neumann, 1902 (cf. paragraph above), Nuttall lot 2882 (BMNH). A male and female from "squirrel", Elolo (= ?Kilelu, near Bio Caboba, Somalia), 1933, presented by Professor E. Stella, lot 17698, Rocky Mountain Laboratory. Two males and 2 females from squirrel, from Nyamyanyango (Eusso Nyiro), Kenya (= B.E.A.), 3 January 1911, Robin Kemp *legit* (BMNH; 1 male and 1 female retained in Hoogstraal collection). A male and female from Voi, Kenya, Robin Kemp *legit* (BMNH).

REDESCRIPTION: MALE: Length from apex of palpi to posterior scutal margin 1.65 mm. to 2.11 mm., width 1.00 mm. to 1.65 mm. *Body* elongate oval, anterior third gradually widening, widest at midlength, lateral margins usually widely convex, broadly rounded posteriorly. *Color* yellowish to dark brown with dark markings bordering palpi, basis capituli, and festoons in some specimens.

Capitulum: Basis capituli dorsally measures 0.14 mm. long, 0.34 mm. wide; surface impunctate or with a few very small, shallow punctations; lateral margins widely diverging anteriorly; posterior margin between cornua straight or very slightly concave; cornua very short, less than one-fourth as long as basis capituli, bluntly triangular, forming about an equilateral triangle. Palpi measuring 0.29 mm. at greatest length; 0.26 mm. at greatest width of single palpus, palpi basally broadly salient, basal margin forming an obtuse angle from insertion to lateral margin; lateral margin regularly and more or less mildly concave; apex moderately rounded; segment 2 about 1.2 times length of segment 3. Palpi ventrally with basal margin forming a narrow, pointed spur about half as long as palpal segment 2; lateral marginal curvature may be like dorsal view or may form a milder arc than dorsally; segment 2 slightly longer than segment 3; segment 3 basally with a robust, tapered, sharply-tipped spur directed slightly internally and overlapping segment 2 for almost its entire length; palpal hairs fine but long, situated as illustrated (Figures 8 and 9). *Hypostome* with shape as illustrated (Figure 6), corona broadly rounded, with minute denticles; dentition 4/4 or 4/3.5, with four to seven (usually six or seven) denticles in each file; denticles broadly ovate, irregular; length of hypostome about 0.15 mm.

Scutum: Lateral grooves shallow, generally extending anteriorly only to midlength of scutum; if longer, becoming even shallower and more inconspicuous anteriorly; posteriorly enclosing first festoon. *Cervical grooves* short, converging, of variable depth. *Scutal surface* flat centrally, downcurved laterally and posteriorly; punctations shallow, mixed small and moderate-sized, distant from each other, numbering from 80 to 110, widely scattered over surface, festoons, and lateral area. *Festoons* clearly marked by deep grooves, length very slightly greater than width; all open anteriorly except anterior pair.

Legs: Coxae each with a conspicuous, pointed, subequal posterior spur, that of I arising flush with inner margin and narrower than others; spurs of other coxae arising near inner margin, basally wider than I; spur I about half as long as coxa, spur II slightly shorter than I, spurs III and IV successively smaller; each coxa with four to six fine hairs; coxal shapes as illustrated (Figure 2). *Trochanter* I dorsally bearing a moderate-sized, pointed shield; ventrally each (except sometimes IV) with a pointed spur becoming successively smaller on posterior trochanters; length of spur I almost equalling trochantal width, shape varying from needlelike to narrowly triangular; spur II about half as long as I and with wider



FIGS. 1–4. *Haemaphysalis calcarata,* (Rocky Mountain Laboratory lot number 17698 from Elolo). 1, male, dorsal view; 2, male, ventral view; 3, female, dorsal view; 4, female, ventral view.

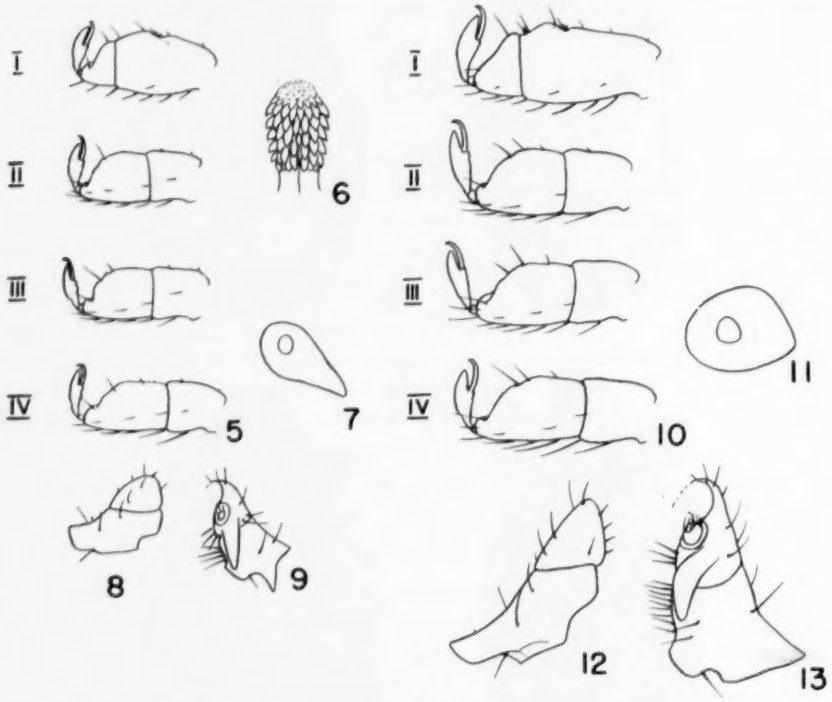
outline, spur III wider and shorter than II, spur IV minute to absent. *Tarsi* stout, abruptly tapered, I slightly wider than long, abruptly tapered from anterior fourth to posterior fourth; II to IV at least 1.5 times as long as wide and with dorsal margin paralleling ventral margin for from half to three-fifths tarsal basal length, thence abruptly tapered to small distal tip; tarsus I with two pairs of ventral hairs, other tarsi with four pairs. *Pads* and *claws* moderate, as illustrated (Figure 5).

Ventral surface: Spiracular plate as illustrated (Figure 7). *Genital aperture* situated

between coxae II, broadly triangular with broadly rounded anterior margin and serrated posterolateral margins. *Anal grooves* as illustrated. *Festoons* conspicuous.

FEMALE: Females readily associated with males; most noteworthy inconsistency is absence ventrally of basal palpal spur (see below).

Capitulum: Palpi measuring 0.42 mm. at greatest length, 0.27 mm. at basal width of single palpus; palpi with same general formation as those of male except that: they are larger and almost twice as long in relation to width; apex is narrower; and basal margin ventrally does not form a spur but either (1) extends in a straight, anteriorly directed line from insertion to lateral junction, or (2) forms a bluntly rounded angle laterad of insertion and thence extends anteriorly to lateral juncture; ventral spur of segment 3 with variable outline but extending only to about midlength or basal third of segment 2. *Basis capituli* short and wide, measuring 0.16 mm. long and 0.47 mm. wide; cornua merely very short, bluntly rounded projections of



FIGS. 5–13. *Haemaphysalis calcarata,* (RML lot 17698 except Fig. 6). 5, Male, tarsi I to IV, lateral view; 6, male, hypostome, ventral view (specimen from Nyamanyango); 7, male, spiracular plate; 8, male, palpus dorsal view; 9, male, palpus, ventral view; 10, female, tarsi I to IV, lateral view; 11, female, spiracular plate; 12, female, palpus, dorsal view; 13, female, palpus, ventral view.

lateral juncture; porose areas shallow, inconspicuous, somewhat indistinct but appearing either oval or almost circular. *Hypostome* (not dissected), appearing wider apically than that of male, with somewhat more regular dentition; dentition 4/4.

Scutum: Length about 0.9 mm., width about 0.8 mm. *Outline* widely diverging on anterior fourth from scapulae to lateral margin; thence straight, very slightly diverging, or slightly converging (depending on degree of engorgement) to about midlength; from midlength more acutely converging to form a moderately broadly rounded posterior margin. *Surface* with moderate number (40 to 60) of well scattered, shallow punctations, mostly large or medium-sized, some small. *Cervical grooves* shallow, slightly converging on anterior third of scutum; even shallower, straight or slightly diverging on middle third, very faint or obsolete on posterior third.

Legs: Coxae with spurs as in male except that these are somewhat wider in outline than those of male, especially on coxa I. *Trochanters* with ventral spur on I slightly overlapping posterior margin of trochanter but shorter and wider than that of male; ventral spur very short to almost absent on II, and indicated only by slight ridge on III and IV. *Tarsi* similar to those of male except that the angles of tapering may very slightly vary.

Ventral surface: Genital aperture forming an elongate, narrow triangle situated between coxae II (engorged specimens). Other characters as illustrated.

Other characters: Characters not mentioned are comparable with those of males.

NYMPH: The nymph was described by Nuttall and Warburton (1915, pp. 443–444, fig. 377), as follows (terminology slightly modified): Scutum subcircular, shagreened, but without definite punctations; cervical grooves well-marked and converging at first, then broad and shallow. Capitulum with base broadening anteriorly, cornua slight; palpi with segment *2* sharply salient laterally, with its posterior border almost straight dorsally but with a ventral retrograde spur directed outward; lateral contour of segments *2* and *3* continuous and concave outwardly; segment *2* twice as long as segment 3; hypostome *2/2*. Legs with sharp internal spur on all coxae.

LARVA: Unknown.

RELATED SPECIES: See this heading under *H. houyi* below.

DISTRIBUTION: (Figure 40): *Ethiopia:* Neumann's (1902) original specimens were stated by Nuttall and Warburton (1915) to be from Sichi Baz, Ethiopia, a now unidentifiable locality. Stella (1940) noted the following Ethiopian locality records in (the then) Galla Province: Mega, Ebano (= El Banno), and Murle; also Caschei which can be now located only at 0°55′ N., 42°38′ E. and Elolo (? = Kilelu, near Bia Caboba) at 41°50′ E., 10°40′ N., both in Somalia. Confusion of localities by Italian workers of this period is common.

Somalia (Italian Somaliland): Nuttall and Warburton (1915) listed Bio Caboba (= Biacobaba); Paoli (1916) (with female illustrations) noted Uanle Uen; Niro (1935) reported Uanle (Uen); Stella (1938) from the Juba (Giuba) River, Stella (1939) without locality, and Stella (1940) from Saha Yero (= Sahaieroi) and Uanle Uen. See also paragraph above.

Kenya: Specimens reported by Lewis (1931) as *H. calcarata* from domestic animals in the Rift Valley are actually *H. parmata* (Hoogstraal 1954B). British Museum (Natural History) collections contain specimens from eastern Kenya, from Voi and from Nyamyanyango (Eusso Nyiro) collected by R. Kemp in 1911.

The inclusion of Egypt in the geographical range of this species (Nuttall and Warburton 1915, p. 508) is undoubtedly a mistake.

Present evidence indicates that *H. calcarata* is confined strictly to East Africa in Somalia, Kenya east of the Rift Valley, and Ethiopia east of the western margin of the Rift Valley.

HOSTS: Wherever hosts of *H. calcarata* have been mentioned in literature or on collection labels, they have been stated either as ground-squirrels or as *Xerus rutilus* subspecies, the only known ground-squirrel in the known range of *H. calcarata* (Figure 40).

HAEMAPHYSALIS HOUYI Nuttall and Warburton, 1915.
Figures 14 to 40

Haemaphysalis calcarata var. *houyi:* Nuttall and Warburton, 1915, pp. 444 to 445; inadequate descriptions of a few superficial comparative characters of male, female, and nymph, from 7 males, 1 female, and 2 nymphs from Bate, New Cameroons,[1] Dr. Houy *legit.* Rousselot, 1951, p. 309, and 1953, p. 119, mentioned specimens from Dakar, Senegal, French West Africa, from *Euxerus erythropus* subsp.; same author, 1953, p. 41, listed Bamako, French Sudan, as collecting locality but omitted record from Senegal (p. 42).

Haemaphysalis calcarata: Neumann, 1910, pp. 173 and 174 described female, with figures 11 and 12, but this is actually female of *H. houyi* (see paragraph above and text below); material based on 8 females from a squirrel, Roseires (= Roseres), Blue Nile, Anglo-Egyptian Sudan, 1906, C. Alluaud *legit* (Paris Museum); 2 associated males, not described, are probably also *H. houyi.* Nuttall

[1] The American Geographical Society and British Museum (Natural History) cannot identify "New Cameroons" or "Bate." The latter may refer to any of the following: in British Cameroons, Badji (approx. 6° 50′ N., 10° 10′ E.), or in French Cameroons, Badé (approx. 4° 24′ N., 14° 33′ E.), Baré (approx. 5° N., 9° 58′ E.), or Basché (approx. 9° 40′ N., 13° 40′ E.).

and Warburton, 1915, p. 444, noted that their Somalia females of *H. calcarata* differed from those described by Neumann, 1910, but did not elaborate.

Haemaphysalis houyi: Hoogstraal, 1954a, p. 307, raised this tick from subspecies to species rank but left details for present report; also noted Sudan distribution by Provinces and that the description of female by Neumann, 1910, who had considered it as *H. calcarata,* was actually that of *H. houyi.*

MATERIAL EXAMINED: Total 170 males, 49 females, 76 nymphs, 3 larvae.

Anglo-Egyptian Sudan (Hoogstraal *legit*): 28 males, 24 females, 33 nymphs, and 3 larvae from Equatoria Province, from *Euxerus erythropus leucoumbrinus* at Kapoeta, Torit, Lamote, and (from *Euxerus erythropus ?laucustris* at) Yei; 10 males, 6 females, 4 nymphs from Bahr el Ghagal Province, from *Euxerus erythropus* subsp., in Galual-Nyang Forest, Gogrial Subdistrict, Tonj District. (Sudan Ghaza Government collection): 2 males from Upper Nile Province, from *"Xerus rutilus"* (undoubtedly a misidentification from *Euxerus erythropus*), from Bor, 21 May 1909, H. H. King *legit.* (British Museum Natural History): 1 male, 1 female from Blue Nile Province at Kamisa, Dinder River, W. P. Lowe *legit.*

French West Africa, Senegal (Hoogstraal collection): 2 males, 1 female from Bignona, Casamance, from *Euxerus erythropus* subsp., Villiers and De Keyser collectors (Gift of Dr. J. Bequaert; others in Museum of Comparative Zoölogy collection).

"New" Cameroons: (British Museum Natural History): 1 COTYPE male from Bate, Dr. Houy *legit* (Nuttall lot 2996).

Uganda: (Sudan Government Collection): 1 male from Atiambo, Lado South, 1 June 1911, H. H. King *legit.* (Onderstepoort collection): 83 males, 10 females, 18 nymphs, from *Euxerus erythropus lacustris* from Kampala, Karamojo, Gulu, and Teso Districts, 1932 to 1952, mostly T. W. Chorley *legit.*

Kenya: (East African Veterinary Research Laboratory collection): 42 males, 17 females, 21 nymphs from "ground squirrels," Kerio, Khalaba (near Bungoma), and near Maseno, 1949 and 1952, W. E. Grainger and K. P. Bailey collectors.

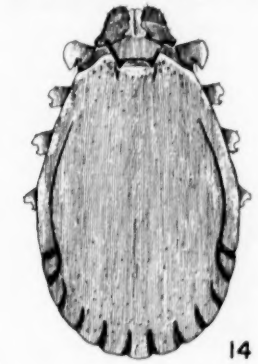
REDESCRIPTION: *Male. Length* from apex of palpi to posterior scutal margin 1.71 mm. to 2.15 mm., width 0.99 mm. to 1.20 mm. *Body* elongate oval, anterior third gradually widening, widest at midlength, lateral margins slightly convex, broadly rounded posteriorly. *Color* yellowish to dark brown with dark markings bordering palpi, basis capituli, and festoons in some specimens.

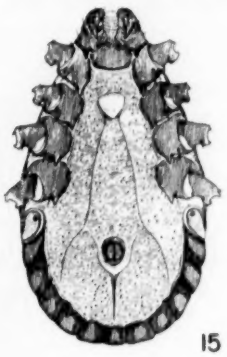
Capitulum: Basis capituli dorsally measures (excluding cornua) 0.15 mm. long, 0.40 mm. wide; surface impunctate; lateral margins widely diverging anteriorly; posterior margin between cornua straight or slightly concave; cornua at least one third as long as basis capituli (in elongate specimens up to one half length of basis capituli), sharply tapering from wide base to usually pointed apex (in short, wide specimens apex may be blunt). *Palpi* measuring 0.30 mm. at greatest length; 0.27 mm. at greatest width of single palpus; palpi basally broadly salient, greatest length about half of total basal width; basal margin forming a more or less obtuse angle; lateral margin more or less concave; apex bluntly rounded, almost as wide as base of segment 3; segment 2 about 1.3 times length of segment 3. Palpi ventrally with basal margin forming a strong, tapered, bluntly-tipped spur from one half to three fifths as long as segment 2 ventrally; segment 3 with a robust, tapered, sharply or bluntly pointed spur directed slightly internally but extending only slightly beyond basal margin of segment 3; palpal hairs fine but long, situated as illustrated (Figures 25 and 26). *Hypostome* with shape as illustrated (Figure 23); corona blunt at apex, with minute denticles; dentition 4/4 in files of six to nine (usually seven) denticles; length of hypostome about 0.18 mm.

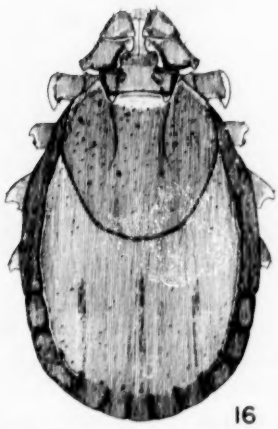
Scutum: Lateral grooves deep, wide, extending to anterior fourth of scutum, posteriorly enclosing first festoon. *Cervical grooves* short, slightly converging, of variable depth. *Scutal surface* usually slightly arched centrally, downcurved laterally and posteriorly; punctations indistinct to almost obsolete, very shallow, mostly small with a few large or medium-sized,

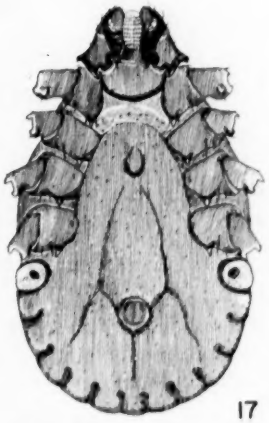
distant from each other, numbering usually less than 20 in all, sometimes with up to 50 mostly small punctations, usually restricted mostly to posterior half of scutum. *Festoons* clearly marked by deep grooves, length equalling or slightly greater than width; all open anteriorly except anterior pair.

Legs: Coxae each with a conspicuous, pointed, subequal posterior spur at or just laterad of the inner coxal margin, spur I about one third coxal length, spurs of other coxae slightly smaller than that of I and of similar shape; each coxa with two prominent, fine white, hairs between spur and outer margin and one shorter hair near inner margin on anterior half (hairs frequently broken); coxal shapes as illustrated (Figure 15). *Trochanter* I dorsally bearing a moderate-sized, pointed shield, ventrally I with a wide, pointed spur either just reaching or very









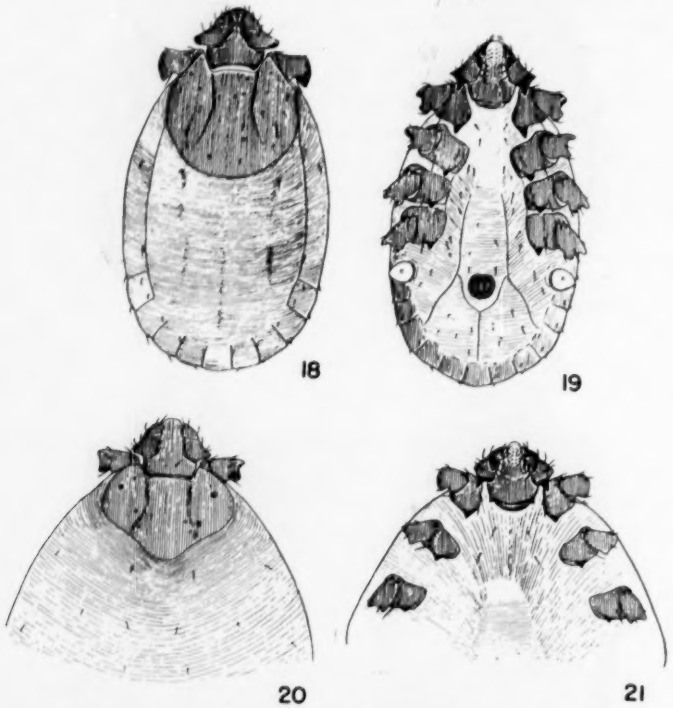
FIGS. 14–17. *Haemaphysalis houyi*, (Anglo-Egyptian Sudan). 14, male, dorsal view; 15, male, ventral view; 16, female, dorsal view; 17, female, ventral view.

slightly projecting beyond posterior margin of trochanter, II with a raised outer spur-like ridge, which does not project beyond margin, III and IV totally unarmed. *Tarsi* stout, abruptly tapered, may or may not be very slightly humped; I slightly wider (1.1) than long, abruptly tapered from anterior fourth almost to apex, II to IV with dorsal and ventral surfaces usually parallel till about midlength (dorsal margin may be slightly convex), thence abrupty tapered to small distal tip; tarsus I with two pairs of ventral hairs, other tarsi with three or four pairs. *Pads* and *claws* moderate, as illustrated (Figure 22).

Ventral surface: Spiracular plate as illustrated (Figure 24). Genital aperture situated between coxae II, broadly triangular with slightly curved anterior margin and serrated posterolateral margins. *Anal grooves* as illustrated. *Festoons* conspicuous.

FEMALE: Females readily associated with males in all essential characters. *Length* (unengorged) and width slightly greater than those of males.

Capitulum: Palpi measuring 0.46 mm. at greatest length; 0.32 mm. at basal width of single palpus; palpi of same general formation as those of male except that: they are larger and dorsally the basal margin forms a more acute sublateral angle, the apex is narrower and rounded, and ventrally the basal margin is produced to form a larger, medially directed spur. *Basis capituli* short and wide, measuring 0.16 mm. long (excluding cornua) and 0.64 mm. wide; cornua broad, about half as long as basis capituli, forming about an equilateral triangle with narrow, pointed or blunt apex; posterior margin between cornua slightly concave; porose areas broadly oval to circular. *Hypostome* (Figure 28), measuring about 0.26 mm. long, dentition 4/4, denticles in files of seven to nine (usually seven); corona moderate, apex slightly concave.



FIGS. 18–21. *Haemaphysalis houyi*, (Anglo-Egyptian Sudan). 18, Nymph, dorsal view; 19, nymph, ventral view; 20, larva, engorged, dorsal view; 21, larva, engorged, ventral view.

Scutum: Length about 1.1 mm., width about 1.0 mm. *Outline* widely diverging on anterior fifth from scapulae to lateral margin; thence slightly diverging to midlength; posterolateral margins strongly converging and posterior margin broadly rounded. *Surface* with few (20 to 30) very shallow punctations of various sizes mostly on anterior half of scutum. *Cervical grooves* of varying depth but usually shallow, forming a slightly concave arc extending to the midlength of the scutum or slightly beyond.

Legs: Coxae differing slightly in shape from those of male (see illustrations) but with very similar posterior spurs and chaetotaxy. *Trochanter* I with raised, triangular, spur not projecting beyond margin of trochanter; II with less well defined spur or none; spurs obsolete on III and IV. *Tarsi* similar to those of male except that each has a more or less pronounced hump dorsally.

Ventral surface: Genital aperture forming an elongate parallelogram situated between coxae II. Other characters as illustrated.

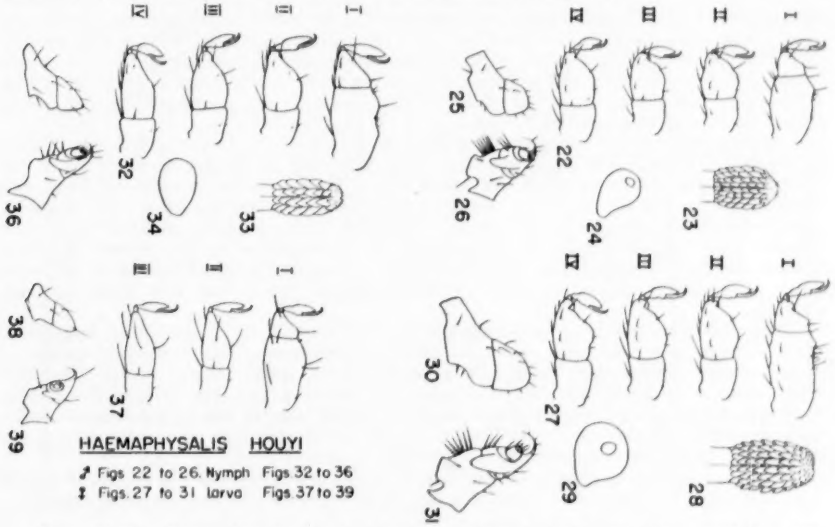
Other characters: Characters not mentioned are comparable with those of male.

NYMPH: Nymphs are easily associated with adults because of similarity in coxal spurs, short and abruptly tapered tarsi, palpal features, converging basis capituli, and faint scutal punctation.

Palpi with shape and outline intermediate between those of male and female but lacking the sublateral angle of the dorsal basal margin, apically somewhat more pointed; ventrally much like female palpi in outline, with a strong basal spur and a strong but very short spur from segment 3. *Basis capituli* with shape as in female but with cornua short and blunt and posterior margin between cornua definitely concave. *Hypostome* with 2/2 dentition in files of seven denticles; corona bluntly rounded.

Scutum subcircular, very slightly (about 1.2) wider than long; punctations ranging from obsolete to as many as 20 very shallow and small ones; cervical grooves converging centrally, extending to posterior fourth of scutum, shallow except anteriorly.

Legs with robust coxal spurs as illustrated (Figure 19); trochantal ventral spurs obsolete or minute on I, absent on others. *Tarsus* I more gradually tapered than in adults, II and III similar to those of female and with small, pronounced dorsal hump, IV more elongate and more gradually tapered than in female. *Spiracle* as illustrated (Figure 34).



FIGS. 22–39. *Haemaphysalis houyi*, (Anglo-Egyptian Sudan). *22*, Male, tarsi I to IV, lateral view; *23*, male, hypostome, ventral view; *24*, male, spiracular plate; *25*, male, palpus, dorsal view (slide); *26*, male, palpus, ventral view (slide); *27* female, tarsi, I to IV, lateral view; *28*, female, hypostome, ventral view; *29*, female, spiracular plate; *30*, female, palpus, dorsal view (slide); *31*, female, palpus, ventral view (slide); *32*, nymph, tarsi I to IV, lateral view; *33*, nymph, hypostome, ventral view; *34*, nymph, spiracular plate; *35*, nymph, palpus, dorsal view (slide); *36*, nymph, palpus, ventral view (slide); *37*, larva, tarsi I. to III, lateral view; *38*, larva, palpus, dorsal view (slide); *39*, larva, palpus, ventral view (slide).

Other characters as illustrated (Figures 18, 19, *32* to *36*).

LARVA: The larva corresponds to adults and especially to nymphs in essential details.

Palpi are like those of the nymph except that the lateral margin is only very mildly concave; ventral spurs are like those of the nymph. *Basis capituli* with shape as in nymph except that the posterior margin is almost straight; cornua very short and bluntly rounded; ventrally, the posterolateral junctures are drawn out to a short, sharp, triangular point. *Hypostome* damaged in available specimens.

Scutum broadly shield-shaped, about 1.5 times as wide as long, outline of anterior half broadly rounded, posterior of midlength abruptly narrowing as a concave arc on either side to the bluntly rounded, narrow posterior margin; punctations shallow, numbering 10 to 14 or so, of mixed sizes, situated mostly laterally; cervical grooves fairly deep and slightly converging anteriorly, posteriorly widely diverging and extending almost to posterolateral margin but very shallow.

Legs with robust coxal spurs as illustrated (Figure 21); trochantal ventral spur obsolete or minute on I, absent on others. *Tarsus* I short, robust, and abruptly tapered; II and III more elongate and gradually tapered from midlength to apex.

Other characters as illustrated (Figures 20, 21, 37 to 39).

DISTRIBUTION: (Figure 40): All collecting localities for *H. houyi* have been listed in this report before the description of this tick. Its known range extends in a belt across the widest part of Africa from the Atlantic Ocean through French West Africa, Cameroons, Anglo-Egyptian Sudan, and Uganda to the northwestern corner of Kenya, west of the Rift Valley.

HOSTS: All authors state either "ground-squirrel" or the specific name of this animal, *Euxerus erythropus* subsp. The only exception is an old Sudan Government collection label reading *Xerus rutilus*, but since these two species have been often confused by non-specialists and the collecting locality is well within the range of *E. erythropus* and 500 miles from the closest known *X. rutilus*, this can be assumed to be a misidentification.

COMPARATIVE DIAGNOSIS AND RELATED SPECIES

Adults of *H. calcarata* and *H. houyi* have the following diagnostic characters in common: palpi of the *H. leachii* type, widely salient, segment 2 with ventral spur, basal margin of male (and female *houyi*) with ventral spur; dentition characteristically 4/4; basis capituli strongly diverging anteriorly; scutum with inconspicuous, shallow punctations; lateral grooves enclosing first festoon; all coxae with well developed, subequal spurs; trochanter I with ventral spur (reduced in female *houyi*); tarsi stout and abruptly tapered; size comparatively small; parasites of African ground-squirrels.

The general facies of both species is so much that of the *H. leachii* group that some specialists have identified specimens, especially lone *H. houyi* females, as aberrant *H. leachii*. The following *H. leachii* characters readily distinguish it from the two species in question: dorsal spur of basal margin of palpi much more developed, no ventral spurs on trochanters, tarsi gradually tapered (except Asiatic *H. leachii indica*), coxal spurs reduced, hypostome often with more denticles (8 to 12) per file and often with more files; combination of other characters in this variable species not equalling the uniform characters of each of the squirrel-parasitizing species. Hosts of African *H. leachii* are never (in my experience), or at least very seldom, ground-squirrels.

The chief characters of *H. houyi* which differ from those of *H. calcarata* are: long lateral grooves, fewer and even more inconspicuous scutal punctations, wider posterior margin of female scutum, considerably larger cornua, smaller coxal spurs, reduced ventral trochantal spurs, presence of ventral spur on basal margin of female palpi; apex of palpi more blunt, male with shorter ventral spur on palpal segment 2, etc. It appears that *H. calcarata* is usually wider in outline than *H. houyi*. As noted elsewhere herein, the hosts and geographical range of each species are distinct from those of the other.

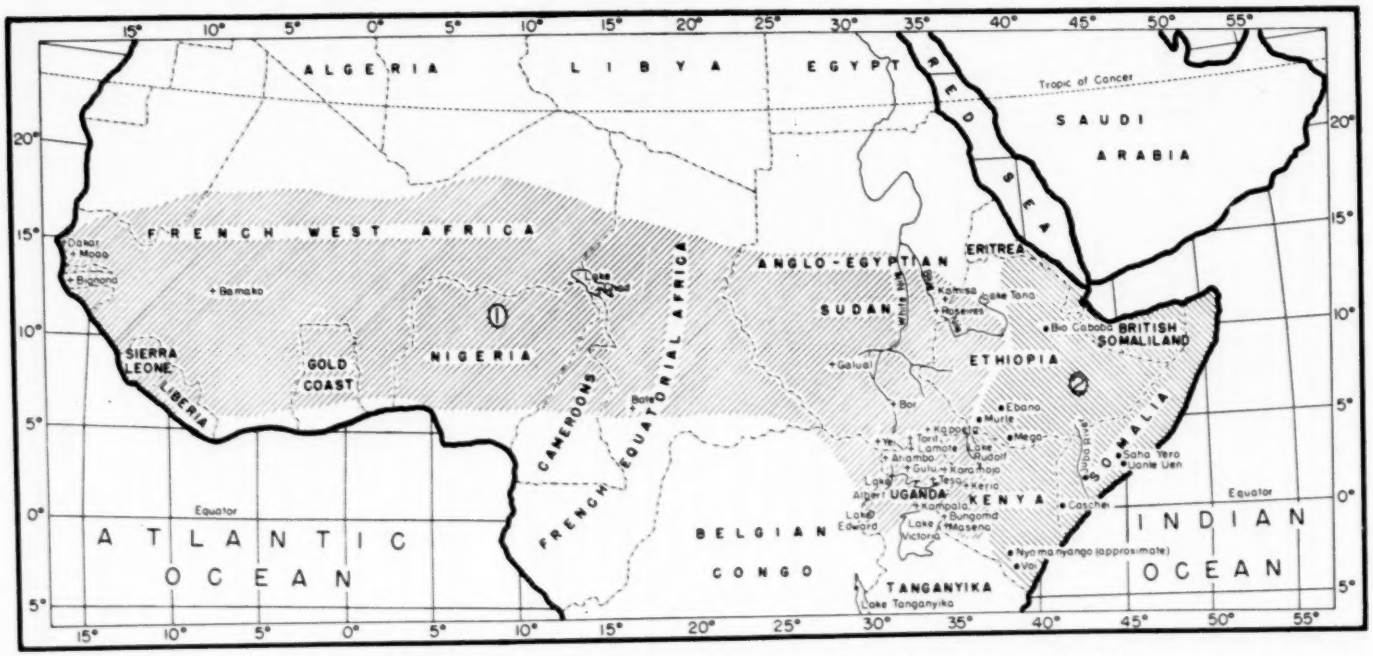
It follows from these criteria that the female "*H. calcarata*" described by Neumann (1910) is so obviously the female of *H. houyi* that no further elucidation of this matter is required.

DISCUSSION AND CONCLUSIONS

Collation of scattered data on *H. calcarata* and *H. houyi*, previously inadequately known and poorly recognized African ticks, reveals the intriguing apparent restriction of these species to separate, adjoining geographical ranges and to separate, closely related species of ground-squirrels whose distribution is the same as that of each tick species.[2]

H. calcarata appears to be confined to East Africa in Somalia, Kenya east of the Rift Valley, and Ethiopia east of the western margin of the Rift Valley. All collecting localities for *H. calcarata* are, faunistically, within the Somali Arid District and the East African Lowland District of the Ethiopian Faunal Region (Chapin, 1932). Wherever hosts of this species have been recorded, they have been stated either as "ground-squirrel" or as *Xerus rutilus*, the only known ground-squirrel in the Somali Arid and the East African Lowland Districts. The host also ranges into the eastern margin of the adjacent Abyssinian Highland District. From

[2] Although there has been some arbitrary choosing among available locations and variations in spelling of tick collecting sites mentioned in literature in this report, all alternates lie within the areas herein specified.



DISTRIBUTION OF GROUND-SQUIRRELS and their TICK PARASITES IN AFRICA

Approximate range of:
Area 1. Euxerus erythropus
Area 2. Xerus rutilus

Collection localities of:
+ Haemaphysalis houyi
• Haemaphysalis calcarata

FIG. 40. Map. Distribution of ground-squirrels and their parasites in Africa.

Legs with robust coxal spurs as illustrated (Figure 21); trochantal ventral spur obsolete or minute on I, absent on others. *Tarsus* I short, robust, and abruptly tapered; II and III more elongate and gradually tapered from midlength to apex.

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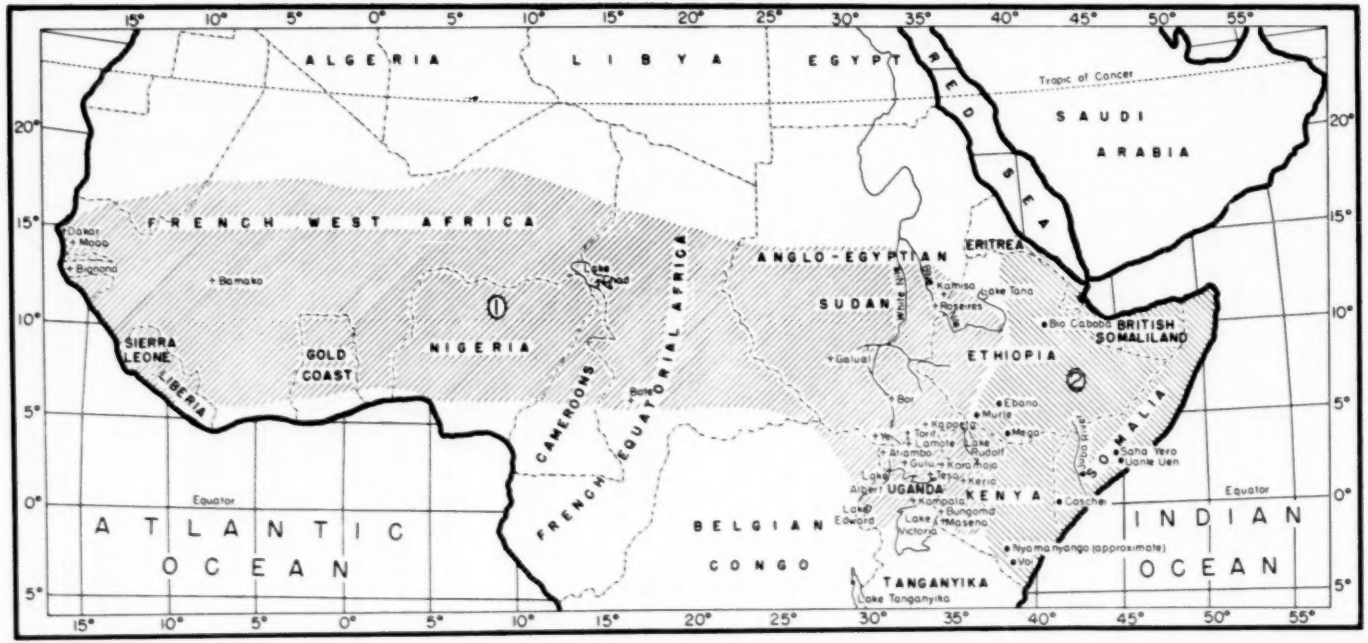

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Approximate range of
Area 1. Euxerus erythropus
Area 2. Xerus rutilus

Collection localities of
+ Haemaphysalis houyi
• Haemaphysalis calcarata

FIG. 40. Map. Distribution of ground-squirrels and their parasites in Africa.

this latter district, which extends both east and west of the Rift Valley, we have no records of the host ranging further west than the Rift Valley, west of which we do know the related ground-squirrel, *Euxerus erythropus,* to be common. Similarly, as already noted, all available records for *H. calcarata* are from localities east of the Rift Valley. It appears, therefore, that the Rift Valley is a physiographic barrier exerting a strong restricting influence on the range of both this ground-squirrel and its specific tick parasite. It should be determined from further collecting whether any of these geographical, faunal, or host bounds are crossed. Present evidence suggests that, if they are, these extensions will prove to be minor and localized.

H. houyi, too, is known only from localities within the range of its apparently specific host, *Euxerus erythropus,* a ground-squirrel extending in a fairly narrow belt across the widest part of Africa from West Africa (north of 5° N.) through the Anglo-Egyptian Sudan and Uganda into those parts of Ethiopia and Kenya which lie west of the Rift Valley. Faunistically, this range traverses several savanna Districts eastward to the western flanks of the Abyssinian Highland District and the East African Highland District (West African and East-South African Subregions). The ground-squirrel's range, the tick's range, and all the faunal districts noted are west of the Rift Valley. The only exception to this statement is the already mentioned Abyssinian Highland District, which does extend east of the Rift Valley but in which the host is found only west of the Rift Valley. It should be noted that wherever *E. erythropus* extends into forested or highland districts, it is, apparently without exception, only in intrusions of savanna between forests or in grassy upland plains with more or less scattered trees.

Additional collecting, especially in the Rift Valley area, will be of biological value in providing more substantial data concerning specificity of these hosts and ticks, influence of faunal regions and physiogeographic barriers, and how specific these inter-relations actually are.

Should future investigations reveal that these ticks and their hosts are infected with disease-causing organisms, further investigations into the inter-relationships of these organisms and the biological factors discussed above suggest themselves for epidemiological consideration.

Because we do not generally expect that even host-specific ticks, as these appear to be, select between two species in the same genus, the apparent specificity of these ticks and their ground-squirrel hosts would seem to be a bolstering argument against lumping *Xerus* and *Euxerus* as one genus, as some mammalogists have recently advocated.

SUMMARY

The previously poorly known African ground-squirrel parasites, *H. calcarata* Neumann, 1902, and *H. houyi* Nuttall and Warburton, 1915, are redescribed to elucidate obvious differences between them and between a ubiquitous tick of superficially similar facies, and known disease transmitting propensities *H. leachii.* The original description of the female of *H. calcarata* is actually that of *H. houyi.* These two species appear to be host-specific, *H. calcarata* on *Xerus rutilus* and *H. houyi,* on *Euxerus erythropus. X. rutilus* ranges in East Africa through Somalia and those parts of Kenya and Ethiopia which lie east of the Rift Valley. *E. erythropus* ranges from the Atlantic coast of Central West Africa through the Anglo-Egyptian Sudan and Uganda to those parts of Ethiopia and Kenya which lie west of the Rift Valley. The inter-relations of ticks, hosts, physical barriers, and faunal districts of Africa are noted. In view of the significant potential of finding disease-causing organisms in these ticks and ground-squirrels, the inter-relations of these biological factors from an epidemiological viewpoint suggests further research. These apparently specific host-parasite relationships suggest the separate generic specificity of each host.

ACKNOWLEDGMENTS

Some years ago Dr. J. Bequaert, Museum of Comparative Zoölogy, told me

that he believed that his West African specimens merited specific rank and presented me with some of his material for comparison with Sudan specimens. The following persons loaned specimens from their collections: Mr. Glen M. Kohls, Rocky Mountain Laboratory; Dr. Gertrud Theiler, Union of South African Veterinary Service; Miss T. J. Walker, East African Veterinary Research Laboratory; Mr. D. J. Lewis, Sudan Government Medical Entomologist; and Mr. E. Browning, British Museum (Natural History). Mr. Browning and Dr. J. May, American Geographical Society, checked localities mentioned in literature and on specimen labels. Dr. H. W. Setzer, U. S. National Museum, furnished the outline of the hosts' range for the map. To all these persons and institutions I am most grateful for their kind coöperation.

ADDENDUM

Since this manuscript was submitted, Dr. J. Bequaert has informed me that Bate, New Cameroons, the type locality of *H. houyi*, is at approximately 6° 40′ N. and 17° E. in savanna country similar to that of Yei, in the Sudan. "Neu Kamerun" was the name given to the German extension of their Kamerun, obtained from the French about 1910, forming two strips reaching the Congo River across French Equatorial Africa. Dr. Bequaert has also seen a male specimen of *H. houyi*, from Mbao, 18 kilometers from Dakar, from *Euxerus erythropus*, 20 February 1947, P. Dekeyser *legit*.

I have seen additional specimens of *H. calcarata* from Gocti, Western Somaliland, from ground squirrels, kindly collected and presented by Dr. A. G. Spooner of the Queen's African Rifles.

Dr. R. B. Heisch of Nairobi, upon reading this manuscript, informed me that in Kenya he and Mr. Grainger have found *Xerus* predominating in the hot, low country of the Northern Frontier District and *Euxerus* in higher arable country from 2000 to 6000 feet elevation, but with few as low as 2000 feet. This corresponds to my own more limited observations in East Africa.

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SOME EGYPTIAN HETEROPHYID TREMATODES*

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University of Southern California,
and

ROBERT E. KUNTZ[1],

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During the early summer of 1953, the senior author was flown to Cairo, Egypt, by the United States Navy[2] to study certain trematodes of medical importance. The work reported here was begun in collaboration with the junior author at the Navy Medical Research Unit-3 and continued during the ensuing year at the University of Southern California.[3] A trip was made to the small fishing villages, Baltim and El Burg, located on the northeastern shore of Lake Burullus where a host of children quickly offered their services for collecting and in a short time they presented us, for a fee, with several thousand snails, *Pironella conica* Blainville, and over one hundred small fishes, *Aphanius fasciatus* (Valenciennes). We are indebted to Dr. T. Abbott, U. S. National Museum, for the identification of the snails, and to Dr. Carl L. Hubbs, Scripps Institution of Oceanography, for the indentification of the fishes. These animals were living in brackish or saline water since the collecting site is near the connection of the lake with the Mediterranean Sea.

The fishes were heavily infected with metacercariae. Some of the fishes were fed to hatchery-raised chicks and when the chicks were examined by the junior author adult heterophyid worms were recovered. Adult worms also were recovered from chicks that were fed experimentally infected *Gambusia affinis*. These adult worms and the larval stages obtained from *Pironella conica* form the basis of this study.

MATERIALS AND METHODS

In early October, 1953, 468 living *Pironella conica* were shipped in damp sphagnum moss from Cairo to the University of Southern California. Of the 215 survivors, 166 were infected with heterophyids. Naturally emerging cercariae were studied either alive to determine the details of the excretory system or as fixed and stained whole mounts. Redial stages, obtained by crushing snails, were studied after fixation and staining. Metacercariae were dissected from formalin-fixed *Aphanius fasciatus*. Adult worms were obtained by feeding naturally infected *Aphanius fasciatus* and experimentally infected *Gambusia affinis* to hatchery-raised chicks. The *Gambusia* were infected in the following way. *Pironella conica*, naturally infected with cercariae of the type described herein as the larval stage

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* The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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[2] Contract Nonr—228(04)

[3] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and University of Southern California, NR 165-252.

of *Stictodora tridactyla*, were separated from those snails infected with other types of heterophyids. Cercariae were pipetted into small crystallizing dishes containing one-half inch of filtered brackish water from Lake Burullus. One *Gambusia, affinis* was introduced into each dish containing the free-swimming cercariae. The *Gambusia*, normally a freshwater species, were previously adapted to brackish water by gradually increasing the salinity over a period of four to six hours. After an exposure period of 1 to 1.5 hours, during which the cercariae were observed to penetrate tissues beneath the scales of the fish, the *Gambusia* were returned gradually to aerated freshwater.

Metacercariae were dissected from the tissues of *Gambusia* four to six days after exposure and were pipetted into the mouths of week-old, hatchery-raised chicks. At autopsy one week later adult heterophyids were recovered from the lower small intestines. Adult worms were fixed, usually in cold Bouin's solution, and stained as whole mounts or were serially sectioned. All whole mounts were stained with paracarmine, serial sections with triosin and hematoxylin.

Some attempts were made to infect killifish, *Fundulus parvipinnis parvipinnis* (Girard), indigenous to Southern California by exposing them to cercariae.

OBSERVATIONS

The adult worms obtained by feeding naturally and experimentally infected fishes to chicks proved to be different from any heterophyids described thus far and hence they are assigned to a new species provisionally placed in the genus *Stictodora*.

Stictodora tridactyla, new species
Adult (Figs. 1, 5)

(All measurements in millimeters)

Diagnosis: Body pyriform, 0.27–0.37 (av. 11 specimens, 0.30) maximum width 0.13–0.19 (av. 0.16). Cuticula spined, spines scale-like, ctenoid, decreasing in size posteriorly. Remnants of eyespots sometimes present at or near level of pharynx. Oral sucker circular and subterminal, 0.024–0.031 in antero-posterior dimension and 0.027–0.031 in width (av. diam. 0.030). Prepharynx 0.009–0.016 in length (av. 0.013). Pharynx cylindrical to globular, 0.024–0.027 in length (av. 0.025) and 0.014–0.019 in width (av. 0.017). Esophagus 0.009–0.047 in length (av. 0.022). Intestinal ceca extending laterally and posteriorly to mid-testicular level. Testes two, oval, at or slightly posterior to mid-body, 0.039–0.069 long and 0.037–0.052 wide (av. 0.051 by 0.045). Vasa efferentia and vas deferens short. Seminal vesicle consisting of three chambers, two large and one small.

Ovary oval, dextral, near and anterior to right testis, 0.034–0.049 by 0.019–0.041 (av. 0.043 by 0.032). Seminal receptacle well-developed, at least as large as ovary, frequently much larger. Oötype small, Laurer's canal not seen, transverse loops of uterus filling much of posterior two-thirds of body. Vitellaria mainly post-testicular, sometimes overlapping testes, but not reaching ovary. Gonotyl and acetabulum enclosed in ventro-genital sac (Fig. 5). Gonotyl approximately 0.031 long by 0.016 wide, provided with three sclerotized processes approximately 0.016 long and armed distally with small spines about 0.001 long. Processes of retracted gonotyl fitting closely, but in extruded gonotyl flaring to produce a tripod effect. Genital pore median, near but posterior to cecal bifurcation. Acetabulum relatively small, attached to wall of ventro-genital sac.

Eggs, in fixed and stained specimens, oval, operculate, yellow, 0.022–0.025 by 0.013–0.016 (av. of 20 eggs 0.024 by 0.014).

Excretory bladder Y-shaped.

Host: (experimental) *Gallus domesticus.*

Location: small intestine.

Types: Holotype No. 37450 U. S. Nat. Mus. Helm. Coll., Paratype No. 532 in Parasitology Collection, Allan Hancock Foundation.

Locality: Lake Burullus, Egypt.

LARVAL STAGES

Snails, *Pironella conica*, collected at low tide along the shore of Lake Burullus, were heavily infected with one or the other of two species of heterophyids. No double infections were observed. Of 215 snails which survived shipment from Egypt to the University of Southern California 166 were infected. The majority of infected snails harbored larvae which one of us (R.E.K.) has found to develop experimentally into adults of a species of *Heterophyes*. Six snails, however, were infected with cercariae of *Stictodora tridactyla*. Measurements of larval stages are in millimeters.

Redia (Fig. 3)

The rediae develop in the digestive gland of *Pironella conica*. They are sausage-shaped or sometimes moniliform with transverse constrictions. Their body length varies from 0.7 to 1.54 (av. 0.88), maximum body width 0.08 to 0.13 (av. 0.11). A birth pore opens near the anterior end of the body. The pharynx is 0.024–0.027 (av. 0.025) in length and 0.019–0.025 (av. 0.023) in maximum width. The gut is short and saccular. Most of the 13 rediae measured contained germinal masses and cercariae, the remainder lacked cercariae.

Cercaria (Figs. 8, 9, 10)

The cercaria is biocellate, monostomate, and parapleurolophocercous. The body cuticle is covered with minute spines, decreasing in size and number posteriorly. In addition, the cuticle bears laterally a few delicate bristles. Body measurements of 26 specimens fixed in cold Bouin's solution without pressure are: length, 0.105–0.130 (av. 0.125); maximum width 0.059–0.076 (av. 0.068). The conspicuous, black eyespots are located approximately one-third the length of the body from the anterior end. Cystogenous glands are located laterally from a little below the eyespots to the posterior end of the body. The mouth is subterminal. Immediately anterior to the mouth is a row of 5–6 elongate spines whose points project toward the mouth opening. Anterior to these are two rows of shorter spines, the first numbering 7–8, the second 8–9 (Fig. 9). These rows of spines are on the ventral side of a protrusible cone. The penetration glands number 14 and their cell bodies are located between the pharynx and the anterior margins of the excretory bladder. The ducts of these glands pass dorsal to the oral sucker in four bundles of 3–4–4–3 ducts to terminate near the anterior end of the body. The cell bodies of the more posterior penetration glands partially surround an oval, heavily-staining mass which apparently is the primordium of the genital system. The oral sucker is oval in outline, 0.022–0.028 (av. 0.025) long by 0.019–0.028 (av. 0.022) wide. The prepharynx is 0.009–0.020 (av. 0.016) long. The pharynx varies from circular to oval in outline. It is 0.006–0.011 (av. 0.007) long and 0.006–0.011 (av. 0.009) wide. Only a little of the esophagus proximal to the pharynx and nothing of the remainder of the digestive tract could be seen. The shape of the excretory bladder varied with the state of contraction from oval, V-shaped, Y-shaped, to four-lobed. The wall of the excretory bladder is composed of cells with granular cytoplasm and relatively large nuclei. The main collecting ducts pass forward from the antero-lateral borders of the excretory bladder to a level somewhat anterior to the mid-body where they divide into anterior and posterior branches. Each of these branches drains three sets of three flame cells each (Fig. 10) so that the excretory pattern

is expressed by the formula $2[(3+3+3)+(3+3+3)]=36$. The caudal excretory tube proceeds a short distance into the tail where it divides into right and left branches which open at the sides of the tail. Usually the caudal excretory duct is not visible until the cercaria has been subjected to cover glass pressure for a few minutes. The tail fits into a socket of the body. The length of the tail is 0.34–0.40 (av. 0.37) and its maximum width is 0.016–0.022 (av. 0.021). These measurements do not include the fins. Both lateral and dorso-ventral fins are present. The lateral fins extend along the anterior two-fifths of the tail. The dorso-ventral fin begins dorsally a short distance anterior to the posterior terminations of the lateral fins and continues around the tip of the tail to terminate on the ventral side very near the level of the posterior margins of the lateral fins. Plicae in the lateral and dorso-ventral fins give the false appearance of supporting structures.

Metacercaria (Fig. 2)

Metacercariae were dissected from naturally infected fishes, *Aphanius fasciatus*, collected at Lake Burullus, preserved in formalin, and shipped to California. Every fish examined was heavily infected with two species of heterophyid metacercariae. For example, a fish of 30 mm. long harbored 302 metacercariae of which 53 are believed to be *Stictodora tridactyla* and the remainder *Heterophyes* sp. The older *Stictodora* metacercariae had the gonotyl developed to essentially the adult condition, making identification easy. One of these metacercariae had even produced embryonated eggs. The *Stictodora* metacercariae were attached loosely to connective tissues in the head and visceral regions of the host while the *Heterophyes* metacercariae usually were embedded in muscles.

The following measurements were taken of 26 fixed and stained *Stictodora* cysts: length 0.13–0.17 (av. 0.15); width 0.11–0.13 (av. 0.12). These measurements include the cyst wall which averaged 0.003 in thickness. In the experimental infections of *Gambusia*, only one-eighth to one-fourth of the penetrating cercariae developed into viable metacercariae.

Heterophyes Larvae

Of the 215 snails, *Pironella conica*, surviving shipment from Egypt to California, 160 or 74 percent were infected with *Heterophyes* larvae. The digestive glands of the snails were infected with the rediae of this parasite. Measurements are in millimeters.

Redia (Fig. 4)

The rediae are elongate and in most respects similiar to the rediae of *S. tridactyla*. A birth pore is present near the anterior end. Measurements based upon 21 stained and mounted rediae are: length 0.31–1.22 (av. 0.91) and maximum width 0.11–0.18 (av. 0.15). The measurements of the pharynx are: length 0.017–0.024 (av. 0.020) and width 0.016–0.020 (av. 0.018). Pharyngeal measurements were more constant than those of redial lengths. The saccular gut is very short. Both cercariae and germ balls were present in all but the smallest of the rediae which contained only germ balls.

Cercaria (Figs. 6, 7, 11)

The cercaria is biocellate, monostomate and pleurolophocercous. The body

cuticle is covered with spines which decrease in size toward the posterior end. A special spined area, located immediately anterior and dorsal to the mouth, consists of three rows of specialized, spear-headed spines. The first row, nearest to the mouth, consists of 3–5, the second row of 7–9 and the third row of 7–9 spines (Fig. 11). The points of these spines are directed toward the mouth and they are supposedly used in the penetration of the second intermediate host and possibly also in leaving the snail host. Body measurements of 25 specimens fixed in cold Bouin's solution without pressure are: length 0.136–0.25 (av. 0.185); maximum width 0.06–0.09 (av. 0.08). The conspicuous eyespots are approximately one-fourth the body length from the anterior end. Diffuse brown pigment is present in most of the body. Cystogenous glands occur in the posterior two-thirds of the body. Penetration glands number 14 and their cell bodies are arranged in two parallel rows extending from a short distance behind the pharynx nearly to the excretory bladder. Each cell body contains coarse granules and a conspicuous nucleus. The ducts of the penetration glands pass dorsal to the oral sucker in four bundles of 3–4–4–3 ducts to terminate near the anterior end of the body.

The oral sucker is circular to oval in outline and measures 0.028–0.037 (av. 0.033) in length and 0.025–0.037 (av. 0.031) in width. It frequently is retracted and has a subterminal opening to the outside. The prepharynx is 0.006–0.030 (av. 0.014) long. The circular to oval pharynx measures 0.006–0.011 (av. 0.008) long and 0.006–0.011 (av. 0.009) wide. The rest of the digestive tract could not be traced. The shape of the excretory bladder varies with the state of body contraction or extension. In extended specimens it is Y-shaped, but during contraction it may be V-shaped or oval and sometimes 4-lobed. The wall of the excretory bladder is composed of rather large cells with granular cytoplasm and large nuclei. The main collecting ducts pass forward from the antero-lateral borders of the bladder to the mid-body level where they divide into anterior and posterior branches. Each branch drains three sets of three flame cells each (Fig. 7) so that the excretory pattern is expressed by the formula $2\,[(3+3+3)+(3+3+3)]=36$. The caudal excretory tube proceeds a short distance into the tail where it divides into right and left branches which open at the sides of the tail. Immediately anterior to the excretory bladder are two heavily staining cellular bodies, presumably genital and acetabular primordia. The tail fits into a socket of the body. The length of the tail is 0.43–0.56 (av. 0.51) and the maximum width 0.022–0.03 (av. 0.025). These measurements do not include the fin which begins dorsally near the proximal end of the tail and continues posteriorly around the tip and then forward ventrally along the terminal third of the tail. The cuticular sheath is dilated and annulated over the proximal one-fourth of the tail. The cortex of the tail is composed of striated muscles and the medulla contains cytoplasm and scattered nuclei.

Metacercaria

The metacercariae are encysted primarily in the muscles of the fish, *Aphanius fasciatus*. Metacercariae dissected from formalin-fixed fishes were easily removed from the cysts. Twenty-six metacercariae removed from their cysts measured 0.14–0.29 (av. 0.22) long and 0.13–0.25 (av. 0.17) in maximum width. The smaller metacercariae still possess eyespots and the cell bodies of penetration glands. The cuticular spines decrease in size posteriorly. The spines are in check rows especially on the anterior half of the body. The oral sucker is almost

completely retracted. The anterior half of the body is richly supplied with tubular glands. Much of the posterior half of the body is filled with the distended excretory bladder. Ovary and testes can be distinguished, and the acetabulum and gonotyl are further differentiated than in the cercarial stage.

Infection Experiments With *Fundulus*

Killifish, *Fundulus parvipinnis parvipinnis,* indigenous to southern California waters, were exposed to both types of heterophyid cercariae. Only the *Heterophyes* cercariae penetrated and encysted. The metacercariae were alive when these infected fishes were fed to chicks. The results were negative so apparently this species of *Heterophyes* will not develop in chicks.

DISCUSSION

The species, *S. tridactyla,* named for the tripod-like gonotyl, is provisionally placed in the genus *Stictodora* because of the short intestinal ceca and the anterior extent of the vitellaria. The ceca, when described in other species of *Stictodora,* reach to or quite near to the posterior end of the body. Witenberg (1953) described a new species, *Stictodora thapari,* from one specimen obtained from a cat in Israel but could not see the intestinal ceca. In this same paper he presented a key for the separation of certain genera of heterophyids including *Stictodora.* He stated that the yolk glands of *Stictodora* are confined to the post-testicular area. In *S. tridactyla* the vitellaria frequently overlap the testes.

Stictodora tridactyla appears to be most like *S. acanthotrema* (Travassos, 1928) Martin, 1950b in regard to the gonotyl. Both have three sclerotized processes on this organ. In the latter species however, these processes are in the form of hooks and apparently do not bear spines. Also in *S. acanthotrema* the intestinal ceca reach nearly to the posterior extremity of the body but in *S. tridactyla* they terminate at the testicular level.

The natural definitive hosts of *S. tridactyla* have not been determined but if this species is like many other heterophyids, it is probable that a variety of fish-eating birds and mammals may serve as hosts. Dr. Anwar Aleem, associate professor of botany, the University of Alexandria, Egypt, stated that small fishes, including *Aphanius fasciatus,* are eaten whole by Egyptians living along the Mediterranean coast. The natives call such food "Bisaria." The fish may be cooked in oil or may be eaten salted and without cooking. The cooking is so brief that the deeper tissues may be raw. Therefore it seems likely that *S. tridactyla* may parasitize man. It is also likely that *Heterophyes* infections in man may be obtained from the eating of this species of fish.

The cercaria of *Stictodora tridactyla* resembles the cercaria of *Parastictodora hancocki* (Martin, 1950b). Similar cercariae have been found in the heterophyid genera *Euhaplorchis* (Martin, 1950a), *Haplorchis,* and *Procerovum* (Hsu, 1951). This seems to further support the suggestion made by Martin (1950b) that these genera should be combined into one subfamily.

SUMMARY

A new species of heterophyid trematode, *Stictodora tridactyla,* is described. Because the ceca are shorter and the vitellaria extend farther anteriorly than in

other species of *Stictodora*, its assignment to this genus is provisional. Adults were obtained by feeding naturally infected *Aphanius fasciatus* collected at Lake Burullus, Egypt, and experimentally infected *Gambusia affinis* (Valenciennes), to hatchery-raised chicks. The worms were recovered from the lower small intestine. Natural hosts probably are piscivorous birds and possibly man. Rediae and cercariae were found in the digestive glands of the snail, *Pironella conica* Blainville, also collected at Lake Burullus. The cercariae are biocellate, parapleurolophocercous, and possess an excretory pattern expressed by the formula, $2[(3+3+3)+(3+3+3)]=36$.

Rediae and cercariae of *Heterophyes* sp. also developing in *Pironella conica* are described. This cercaria is biocellate, pleurolophocercous, and possesses an excretory pattern expressed by the formula, $2[(3+3+3)+(3+3+3)]=36$.

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Explanation of Plates and Figures

(All drawings made with the aid of a camera lucida except Fig. 5)

Abbreviations

A acetabulum; B excretory bladder; C cystogenous gland; E eyespot; G gonotyl; GP genital primordium; I intestine; M mouth; O oral sucker; OV ovary; P pharynx; PG penetration gland; R seminal receptacle; S seminal vesicle; T testis; V vitellaria.

Plate I

Fig. 1. Dorsal view of *Strictodora tridactyla* adult from experimental infection in chick.
Fig. 2. Metacercaria of *S. tridactyla* removed from fish, *Aphanius fasciatus*.
Fig. 3. Redia of *S. tridactyla* removed from the digestive gland of the snail, *Pironella conica*.
Fig. 4. Redia of *Heterophyes* sp. removed from the digestive gland of *P. conica*.
Fig. 5. Diagram of portion of the reproductive system and acetabulum of *S. tridactyla*.

Plate II

Fig. 6. Cercaria of *Heterophyes* sp.
Fig. 7. Excretory system of *H.* sp. cercaria.
Fig. 8. Cercaria of *Stictodora tridactyla*.
Fig. 9. Spines associated with mouth of *S. tridactyla* cercaria.
Fig. 10. Excretory system of *S. tridactyla* cercaria.
Fig. 11. Spines associated with mouth of *Heterophyes* sp. cercaria.

PLATE I

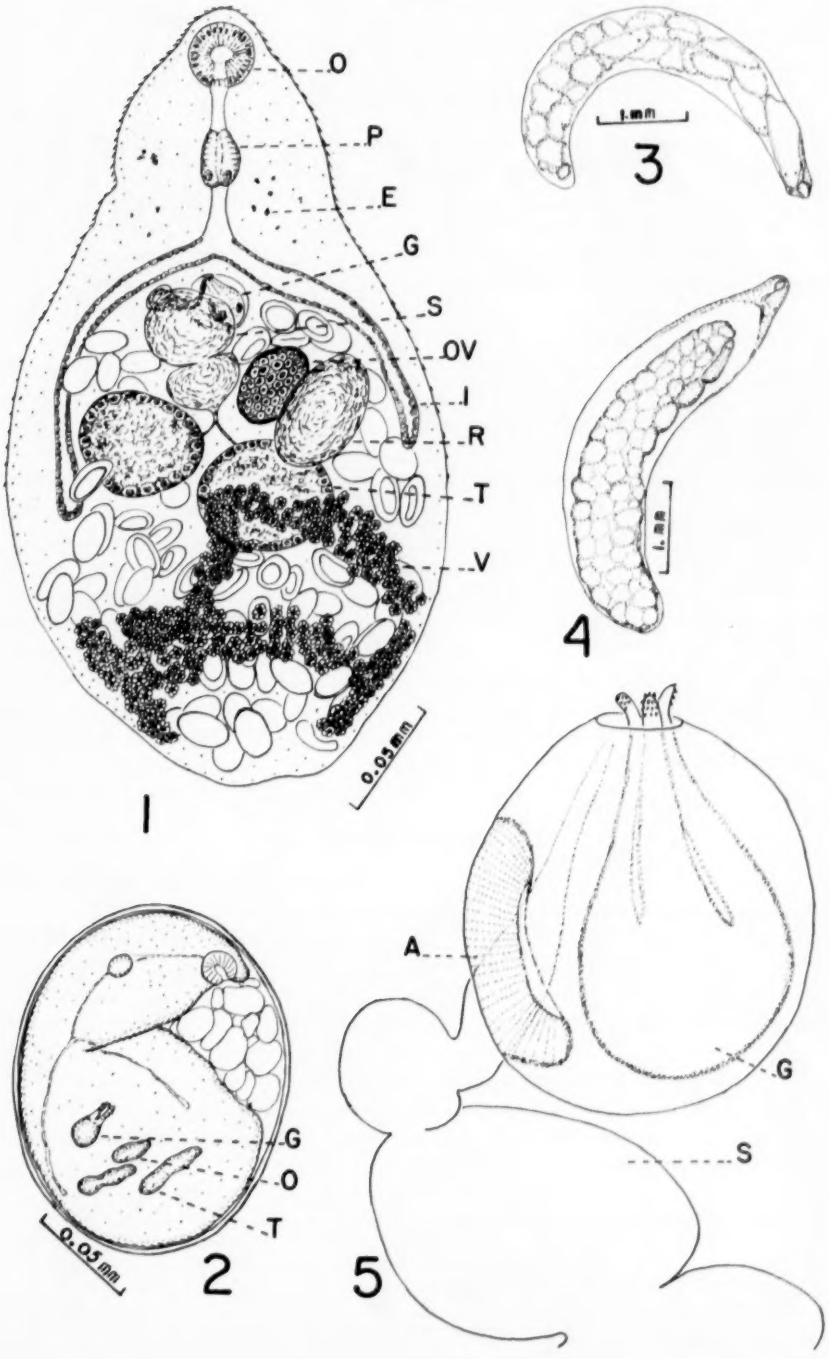
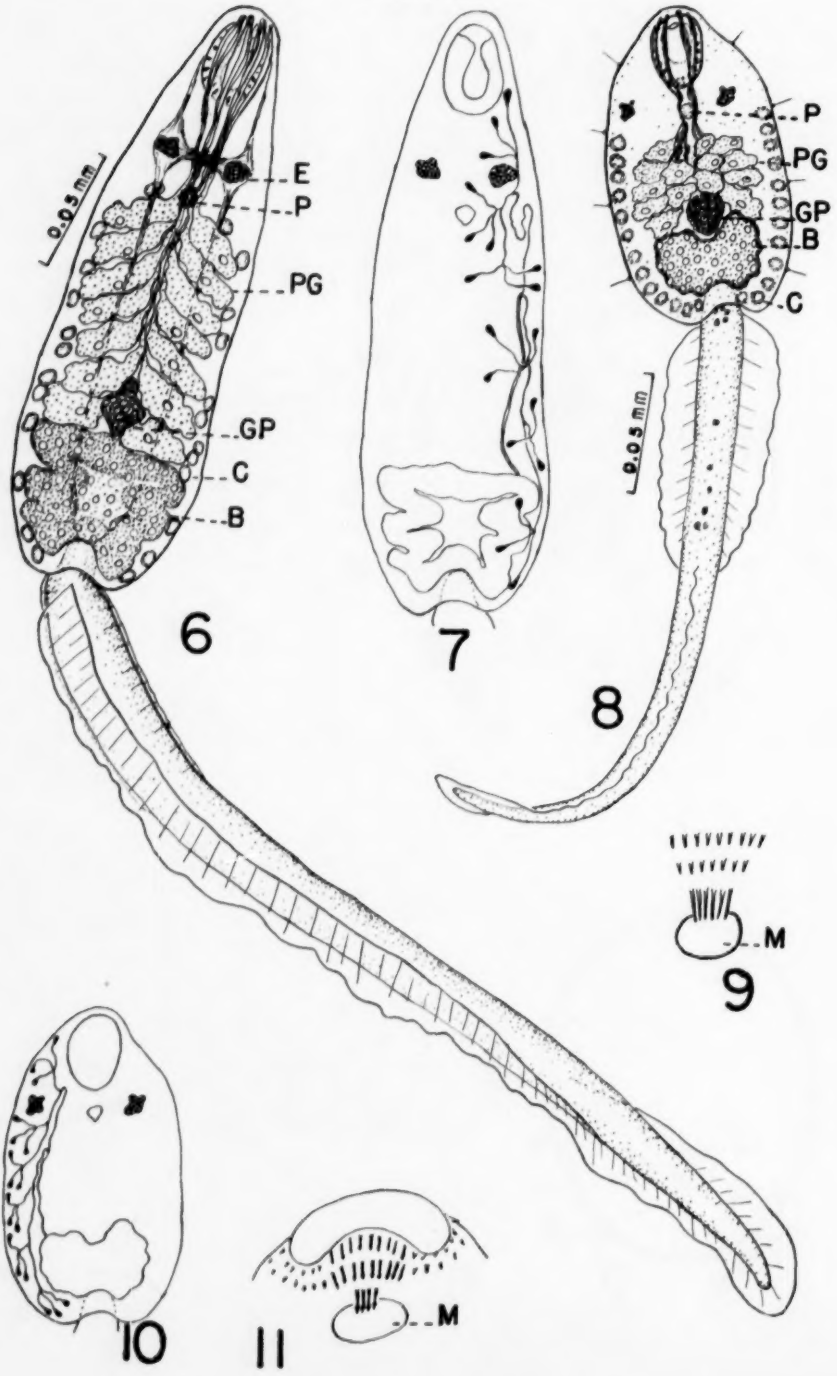


PLATE II



THE LIFE CYCLE OF *ISOSPORA FELIS* IN THE KITTEN

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von Wasielewski (1904) first described *Isospora felis* under the name of *Diplospora bigemina* in a detailed account of the formation and sporulation of the oocysts and morphology of the schizonts and free merozoites in the cat.

Under the name of *I. bigemina* Swellengrebel (1914) gave a description of the development of *I. felis*. For the first time he described the schizogonous cycle in the epithelial cells of the small intestine, and the formation of the microgametocytes, macrogametocytes, and oocysts.

Wenyon (1923) proposed the name of *I. felis* for the coccidium in the cat producing the oocysts largest in size. He described and presented pictures of the developmental stages of the asexual and sexual cycles from scrapings of the epithelial cells of the villi of the small intestine and cecum from a natural infection of *I. felis*.

Artificial infections of *I. felis* were studied by Andrews (1926) in 4 week old kittens given 100,000 sporulated oocysts. He described the symptomatology, but had no discussion of the morphological stages.

The present study follows Andrews's work in that experimental infections of *I. felis* were produced in young kittens. The symptomatology was noted, but more emphasis was placed on the morphological stages found in the life cycle, organs parasitized, and the location of the coccidial stages in the different intestinal tissues.

The purpose of the present investigation was: (1) to study microscopically and describe the morphological stages in the life cycle of *Isospora felis* in kittens artificially infected with this single species of coccidium, (2) to establish the number of schizogonous and sporogonous cycles, (3) to determine the prepatent period, and (4) to observe the organs parasitized.

MATERIALS AND METHODS

Care and management of the kittens.

The kittens used in this study were obtained from private sources in the area of Lansing and East Lansing, Michigan. They ranged in age from 28 to 60 days (average 43.3 days) when received. They were weaned and less likely to be naturally infected with coccidia at this age. Kittens which are infected or have recovered from *Isospora* spp. infections are completely immune to reinfection (Andrews, 1926).

On arrival each kitten was given 2 ml. of feline distemper vaccine supplied by Pitman-Moore Company, Indianapolis, Indiana, and isolated from the colony for 7 days. As soon as feces were available, a total of 12 fecal examinations were done on each kitten by the centrifugal concentration procedure using saturated sugar

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solution. Since the prepatent period had been established for *I. felis* in the kitten as 5 to 6 days (Andrews, 1926), 12 pre-infection fecals were chosen to be examined, and they covered a period twice as long as he had indicated. Only those kittens which did not show oocysts in the feces were used in this study. Andrews may not have ruled out natural infections of his four week old kittens though this seems unlikely in view of the fact that his animals were born in the laboratory of unparasitized mothers and precautions were taken to prevent their becoming infected. A natural incidence of *Isospora* spp. of 89% was found in some Michigan kittens (Hitchcock, 1953). Of the 304 kittens examined, the 19 used in this study were found free of coccidia and were given the second 2 ml. of vaccine 7 to 10 days following the first vaccination. Of the 304 kittens 144 were given a second dosage of vaccine and were used in an earlier work (Hitchcock, 1953) as they were found to be naturally infected with coccidia. Eleven of the twice vaccinated kittens died but only two were proven to have distemper. This prophylactic program seems desirable to maintain a colony of kittens for research purposes.

All cages were washed and disinfected daily. Extreme precaution was taken in the care and management of the kittens to prevent the introduction of coccidiosis.

The food, consisting of canned cat food ("Kalico" and "Three Little Kittens"), dried dog meal ("Zinn's Peerless") and whole, pasteurized, homogenized milk, was fed twice daily in clean sterile Petri dishes.

Experimental infection.

The oocysts of *I. felis* used for infecting the kittens were obtained by the sedimentation method from kittens with single natural infections. Fecal samples collected from these carriers were repeatedly concentrated by the sugar flotation method and examined microscopically for the presence of mixed oocysts. Feces containing only oocysts of *I. felis* were concentrated and sporulated to be used for the infective material.

During the microscopical examination of the tissue sections no parasitological stages of coccidia other than *I. felis* were found, which helps to substantiate the evidence that the oocyst suspensions used for infecting contained only *I. felis*.

These oocysts were sporulated in 2.5% potassium dichromate solution. A constant supply of air was bubbled through the oocyst-dichromate mixture for 72 hours at 20 to 22°C.

Each kitten was infected with 100,000 sporulated oocysts given orally in 1 ml. of water. The number of oocysts per ml. was determined by taking the average of the number of oocysts counted in four different 0.01 ml. samples. The dosage of oocysts of *I. felis* was established at 100,000 per kitten, as this amount had proved fatal in kittens four weeks old (Andrews, 1926). Thus it was chosen as the dosage which would probably not kill animals approximately nine weeks old. When inoculated the kittens ranged in age from 49 to 80 days (average 65.3 days) and the weights ranged from 581 to 1,152 grams (average 866 grams).

Fecal examinations of each animal were done daily, when the samples were available, until it was sacrificed.

Autopsy.

The infected kittens were killed on the 2nd, 3rd, 4th, 5th, 6th, 7th or 8th days after inoculation. Representative tissues were removed from the junction of the small

intestine and the stomach, at the first two inches of the small intestine and every three inches of the remainder of this organ; there being a total of 11 samples. The cecum was cut into four pieces. Similar portions were removed from each 1.5 inches of the large intestine.

Two kittens, which did not show oocysts in the feces after 12 fecal examinations, were sacrificed and the above mentioned tissues removed for staining. These pieces were used for the study of the normal histology of the intestinal tract.

The portions of the intestine for the microscopical examination were fixed in Zenker's solution, infiltrated with paraffin, sectioned at a thickness of 7 microns and stained with the routine eosin hematoxylin staining technique.

Scrapings were obtained from four areas of the fresh mucosal lining of the small intestine, placed in physiological saline, and examined microscopically while warm for oocysts and other Protozoa. Any gross lesions were noted.

The entire stained cross-sections were systematically examined using the oil immersion objective. Two or three kittens were used for each time interval and 22 pieces of the small intestine, two of the cecum, and two of the large intestine of each animal were examined. All the different morphological stages of *I. felis* for each kitten sacrificed at the mentioned time intervals were drawn as they appeared in the tissues.

RESULTS

Two kittens, not showing oocysts in the feces after 12 fecal examinations had been made over a period of at least 12 days, were sacrificed and the stained cross-sections of the tissues were used to study the normal histology of the coccidia-free animal before the infected hosts were examined.

The main difference in the tissues of the infected and non-infected animals was the presence of the intracellular stages of *I. felis*. The parasitized epithelial cells were at times greatly enlarged, distorted and the nucleus crowded to the side. At times these normal tissues were referred to during the observations of the infected portions of the gut.

Two kittens were sacrificed two days after being infected with 100,000 sporulated oocysts of *I. felis*. The morphological stages of the coccidia found are called first generation schizonts. These oval or round stages vary in size from 4 to 7 microns in width to 10 to 16 microns in length depending on the stage of development. In the immature schizont (Figs. 1 and 2) the chromatin material appeared to occur in large irregular clumps which became smaller and retained their irregular shape as the schizont matured (Figs. 3 and 4). The chromatin particles, about 1 to 2 microns in size in the mature schizont, were not associated with definable cytoplasm. The number of chromatin masses, called first generation merozoites, varied between 40 and 60 in number and were difficult to count accurately. No first generation merozoites were recognized free in the lumen of the gut, although mature first generation schizonts were observed opening into the lumen of the small intestine.

Both immature and mature first generation schizonts parasitized only epithelial cells of the small intestine. No coccidial stages were found in the large intestine and the cecum. In one kitten first generation schizonts were found in all sampled intervals, except the first 2.5 inches of the small intestine. In the other kitten no

coccidial stages were found until the middle portions of the small intestine were examined.

At this early time in the life-cycle, the immature first generation schizonts seemed to be more prevalent than the mature first generation schizonts.

Immature and mature first generation schizonts were found throughout the small intestine and cecum of the two kittens sacrificed three days after infecting with *I. felis* oocysts. These stages appeared similar to those previously described. In the immature first generation schizont (Fig. 2) some of the chromatin masses seemed to be in vacuoles. The mature first generation schizonts (Figs. 3 to 5) had first generation merozoites as described in the animals killed at two days.

In the small intestine of one of the kittens, 25 inches from the stomach, stages of the second asexual or schizogonous cycle represented by an immature second generation schizont (Fig. 12) were found. The nuclei had divided, but not the cytoplasm. The nuclei seemed to be in vacuoles and no karyosomes were observed. Three mature second generation schizonts were found in the small intestine next to the stomach. Each of the 14 enclosed second generation merozoites measured 3 to 5 microns. The nuclei appeared to be in vacuoles and surrounded by a small portion of cytoplasm. The majority of the stages found at this time were immature and mature first generation schizonts. Very few second generation schizonts were found.

In these hosts sacrificed four days after infection, morphological stages of the first schizogonous cycle were similar to those previously described. Immature first generation schizonts and mature first generation schizonts were found.

In only one of the three kittens examined were second generation asexual stages found. A second generation trophozoite with a single nucleus, 2 microns in diameter, and set in a vacuole is delineated in Figure 6. The nucleus of the host cell was crowded to one side. The second generation trophozoite divided, forming two crescent shaped merozoites, each with its own nucleus and cytoplasm (Fig. 7).

The first generation schizonts were found throughout the cecum and the small intestine, whereas the second generation trophozoites and schizonts were found in the small intestine starting ten inches from the stomach. The majority of the coccidial stages found on the fourth day were first generation asexual stages. Only a few second generation asexual stages were found in one of the kittens examined. Immature and mature first generation schizonts were found in both kittens throughout the small intestine five days after infecting, but not in the cecum or large intestine. These stages appeared similar to those schizonts previously described.

Second generation asexual stages were represented by forms in various degrees of schizogony. The second generation trophozoite (Fig. 6) divided forming the immature second generation schizont (Fig. 7) containing two adherent, crescent-shaped merozoites. In Figure 8 is shown a second generation schizont in which the merozoites appeared as cones, the head portion being the nucleus surrounded by a vacuole and the pointed portion the blue cytoplasm. Further division took place with the formation of an immature schizont with four merozoites (Fig. 9) and then 6 to 8 merozoites (Figs. 10 to 13). No second generation schizonts with more than 16 merozoites were found. Free second generation merozoites were found in the lumen of the small intestine (Fig. 17). The second generation asexual stages were found only in the small intestine and predominated over the first generation asexual stages. No sexual stages were observed.

The first generation asexual stages observed in the animals killed six days after infecting were as previously described. These stages were decreasing in number and were superseded by the second generation asexual stages. The second generation trophozoite (Fig. 6) divided forming an immature second generation schizont (Fig. 8) with two merozoites. Each of these merozoites divided resulting in four merozoites (Fig. 9). By repeated division immature second generation schizonts containing 6 and 8 merozoites were found (Figs. 10 to 13) which matured into second generation schizonts containing 12, 14, 16, 20, and 24 merozoites (Figs. 14 to 16, 30 and 31). A second generation schizont (Fig. 9) was found free in the lumen of the small intestine. Both asexual generations were distributed throughout the small intestine, but a few second generation trophozoites and immature schizonts were seen in the cecum and large intestine of one of the kittens.

One sexual stage, a mature macrogametocyte (Fig. 19), was found in the epithelium of the small intestine approximately 27 inches from the stomach. The parasite was irregular in shape and contained a large, dark, central nucleus in a vacuole and the cytoplasm stained an uneven pale blue.

In the three kittens sacrificed seven days following infection, a few immature and mature first generation schizonts were distributed throughout the small intestine, while none was noticed in the cecum and large intestine.

The second generation asexual stages were found in the lower two-thirds of the small intestine in the three animals. In one kitten, two second generation trophozoites were found in the cecum, but no parasites were observed in the large intestine. The second generation trophozoite, as previously mentioned, divided forming the immature schizont. Many of the mature second generation schizonts contained 10, 12, 18, 20 and 24 merozoites. The second generation asexual stages predominated over the first generation asexual and sexual stages.

Sexual stages were found in the small intestine of one of the three kittens. This host passed oocysts in the feces on the day of sacrifice and oocysts were found in the fresh mucosal scrapings. The immature macrogametocyte (Fig. 18) resembled the second generation trophozoite, but was larger. The large, dark nucleus in a clear area was either central or eccentric, and the cytoplasm contained darker staining masses. By a process of growth, the macrogametocyte (Fig. 19) was formed, which was larger than the immature stage and possessed a large nucleus in a vacuole and finely stained particles in the cytoplasm.

Only later stages of the development of the microgametocyte were observed at this time. The immature microgametocyte (Fig. 21) showed peripheral chromatin masses, invagination of the cytoplasm, and as it developed there appeared a residual mass of cytoplasm with scattered chromatin and small, crescentic, nucleated bodies, the microgametes (Fig. 22). The mature microgametocyte (Fig. 23) lost the residual mass and was filled with numerous microgametes.

The oocysts in the epithelial tissues (Fig. 24) possessed a dark, central nucleus, cytoplasm with darkly stained scattered masses and a thick, unstained hyaline wall.

Many immature macrogametocytes, a few mature macrogametocytes, a very few microgametocytes and intracellular oocysts were noticed on the seventh day.

At the time of autopsy, oocysts were present in the scraping of the wall of the small intestine of only one of the three kittens. Stages of the first generation asexual cycle were not noted in either of these kittens killed on the eighth day after inoculation.

The second generation trophozoites and schizonts were found in one kitten throughout the small intestine and in the other in only the lower two-thirds of this organ. In this latter host, second generation schizonts with from 2 to 8 merozoites were found in the cecum and one second generation trophozoite was seen in the large intestine. Many second generation schizonts with more than 12 merozoites were observed in the small intestine of both animals (Figs. 14 to 16).

Sexual stages were found throughout the small intestine of one kitten and in the lower two-thirds of this organ of the other. One macrogametocyte was found in the cecum of the latter host, although no sexual stages were found in the large intestine. The immature macrogametocyte (Fig. 18) with coarse chromatin masses in the cytoplasm, developed into a mature macrogametocyte (Fig. 19) with fine chromatin particles.

No differences were observed to suggest that the early stages of microgametocytes (Fig. 18) could be distinguished from comparable stages of macrogametocytes. The nucleus fragmented further resulting in a more developed microgametocyte (Fig. 20). Indentation of the cytoplasm and collection of the chromatin on the periphery of the parasite occurred (Fig. 21). As the parasite enlarged, the cytoplasmic mass was reduced and the microgametes formed (Fig. 22). The mature microgametocyte (Fig. 23) had no cytoplasmic residual mass and was filled with numerous microgametes which were curved, nucleated structures measuring 1.5 by 6 microns, and too numerous to count. Free microgametes in the lumen were not seen.

An oocyst within the epithelial cell is presented in Figure 24. Unsporulated oocysts were passed in the feces of one of these kittens on the seventh day and the other on the eighth day, thus the prepatent period is 7 to 8 days. Unsporulated oocysts were found in the fresh mucosal scrapings from the small intestine of both animals.

Of the 18 kittens infected with 100,000 sporulated oocysts, none showed gross or microscopic blood in the feces. Some of them had diarrhea, as did some of the non-infected controls. The animals were alert, playful, ate well and showed no symptoms which were attributed to the infection with *I. felis*. No gross or microscopic lesions, or deaths, were encountered.

DISCUSSION

Most of the early studies on *I. felis* were made on natural infections instead of experimentally produced conditions. Grassi (1882) failed in his attempt to infect cats with oocysts of *"Coccidium Rivolta"* which had "developed a few days." Andrews (1926) produced artificial infections by *I. felis* in four-week old kittens (750 grams or less). He found that if he gave less than 100,000 sporulated oocysts by stomach tube the kittens lived, but if he gave over that number they died within a week.

In the present study, kittens of approximately the same age were infected, pre-infection fecal examinations were done to establish that the hosts were coccidia free, all animals received the same number of oocysts of *I. felis*, and post-infection fecal examinations were made until the animals were sacrificed. Two control kittens were observed and killed after 12 daily fecal analyses showed no oocysts of coccidia.

The kittens sacrificed at the end of two days showed only immature and mature first generation schizonts. Representative mature first generation schizonts in the epithelial cells of the small intestine are shown in Figure 25. Stage B is about ready to release the first generation merozoites into the lumen of the gut. The merozoites appear to be mainly chromatin with no definable cytoplasm. Stage A is situated further below the cilia. No trophozoites (stages with a single chromatin mass) were found. The reason for this may be that no kittens were sacrificed earlier than 48 hours after infecting.

The earlier workers, Swellengrebel and Wasielewski, and Wenyon as late as 1923, did not use the term "trophozoite" to designate the stage with only one chromatin mass. The smallest form, which Wenyon described in the epithelial cells as the "schizont," would, in the light of our present knowledge, be called the second generation trophozoite.

The first generation schizonts were not mentioned by the early investigators. This might have been due to the fact that most of the earlier observations were made on natural infections late in the cycle, after oocysts were being passed in the excreta, and the first generation asexual cycle would have been completed before this time.

Wasielewski (1904) portrayed, under the name of *Diplospora bigemina, I. felis* as "twin forms," diameter 10 microns, average 7–8 microns or 9–10 microns, which could be confused with fat. He had possibly seen second generation schizonts with two merozoites. Their outlines were very dark and marked. The contents were frequently collected in the center and consisted of a granular mass which was arranged in one or several bodies. At times one "twin body" could be surrounded by an envelope, but there were usually two in the membrane. At autopsy he found the parasite irregularly distributed in the intestinal epithelium and even in the depth of the mucosa arranged singly or in groups. Since he found parasitic stages in the depth of the epithelium, Reichenow (1921) was probably correct in assuming that Wasielewski was studying a mixed coccidial infection.

Asexual tissue stages of *I. felis* were outlined by Swellengrebel (1914) under the name of *I. bigemina*. He fixed smears of the small intestine of cats with natural infections in $HgCl_2$-alcohol and stained with Delafield's hematoxylin, and also embedded tissues in paraffin and cut sections at 10 microns. He described stages which, when developed to the size of 12 microns, had nuclear division into two pieces, and became smaller and compact with the clear zone less distinct. The protoplasm was alveolar, homogeneous, divided and light lines appeared between protoplasmic divisions. This stage, in the light of the present work, would now be called the immature second generation schizont. He stated that the parasite increased in size, filled the host's cell and pushed the nucleus to the side, but did not rupture the host cell's membrane. The stages which he characterized were uneven in size, 20 by 25 microns and 32 by 48 microns. This compared with the mature second generation schizont of the present study. Merozoites, 8–12 microns by 3–4 microns, were sketched as free in the lumen of the small intestine. The nucleus was a compact structural, chromatin mass surrounded by a light zone. Thus, Swellengrebel was the first to relate the morphological characteristics of the second generation asexual stages.

The second generation trophozoite was described by Wenyon (1923) under

the name of "schizont." He sketched these stages, 5 microns in length, as being the smallest forms found lying in vacuoles in the epithelial cells. Their nuclei were spherical with a definite membrane, and the parasites were curved with a pointed anterior end and a rounded posterior end. This organism grew until it was about 5 by 10 microns and then nuclear division commenced. It was ovoid in shape by the time eight merozoites were formed in the mature second generation schizont. These merozoites developed into either schizonts or sexual stages. Wenyon related, under the heading "schizont," a discussion of the morphological characteristics of the second generation trophozoite and schizonts, since he did not observe the first generation asexual stages.

The majority of the stages found in the present investigation in kittens sacrificed at the end of three days were immature and mature first generation schizonts. A very few second generation schizonts were observed. Not all of the first generation merozoites penetrated the host's cells at the same time, nor did they all grow at the same rate. A very few of the first generation merozoites entered the second asexual cycle before the others, thus accounting for the early second asexual stages on the third day of the life cycle of *I. felis*.

The first asexual generation stages were still more prevalent than the second asexual generation at the end of four days. More of the second generation stages were found at this time than at the three day period. This finding of a few stages of the next generation before the first cycle is completed is not unusual as it had been observed in the coccidia of other animals. The second asexual generation kept increasing in numbers and eventually dominated the scene.

By the end of the fifth day after infection, the second generation asexual stages predominated over the first. Second generation trophozoites and immature schizonts with 2 and 4 merozoites were the most common. The second generation trophozoite (Fig. 26-C) is cone-shaped, lying in a vacuolated epithelial cell of the small intestine. The host cell's nucleus has been pushed to the right. This trophozoite divided into two merozoites (Fig. 27-D) which have pushed the host's cell nucleus to the lower right. Further division of the chromatin and cytoplasm took place with the formation of pairs of merozoites. An immature second generation schizont with four such pairs of merozoites is shown in Figure 28-E; those on the left are the clearest. A few immature schizonts with 12 and 16 merozoites were observed.

For the first time in this study, free second generation merozoites were found in the lumen. These stages entered epithelial cells and probably repeated the second generation asexual cycle or initiated the sexual cycle.

At the end of the sixth day after infection, the second generation asexual cycle definitely predominated. Many second generation trophozoites, many immature schizonts with 2, 4, 6, 8, and 10 merozoites, and a few schizonts close to maturity with 12, 14, 16, and 24 merozoites were represented. An immature second generation schizont with 6 merozoites, which are not all in focus in one plane, is represented in Figure 29-F. Further along in the development is Figure 30-G which is larger and contains 14 merozoites. The host cell's nucleus has been pushed to the lower left of the cell. A mature second generation schizont (Fig. 31-H) with 24 or more merozoites has greatly enlarged the invaded epithelial cell. Many immature second generation schizonts were present. These stages represented dividing forms which resulted in maturing schizonts with 12, 14, 16, 24, or more merozoites.

Only one sexual stage, a mature macrogametocyte, was observed in these kittens. This was the first indication that the bulk of the sexual cycle probably would appear on the seventh day. The absence of oocysts in the mucosal scrapings of the small intestine and excreta furnished evidence that the sexual cycle had not advanced to the stage of oocyst production.

Swellengrebel (1914) listed the mature schizonts as measuring 20 by 25 microns and 32 by 48 microns. None of the second generation asexual stages measured by the author were more than 25.5 microns by 31.5 microns. Wenyon (1923) expressed the opinion that 8 merozoites were the usual number and that 16 merozoites rarely occurred. In the present study, second generation schizonts with 10, 12, 14, and 16 merozoites were common and those with 20 and 24 were noticed in several instances. Wenyon did not relate the sizes of the immature and mature schizonts.

Three kittens were examined at the end of the seventh day, since only one sexual stage was found on the sixth day. Oocysts were found in the mucosal scrapings and in the excreta of one of these animals. Sexual intracellular stages and many second generation asexual forms were found in the small intestine.

At the end of the eighth day, the second generation asexual and sexual stages predominated. Second generation schizonts with from 2 to 24 merozoites were seen.

Most of the sexual stages were found in the small intestine. Many immature macrogametocytes, a few mature macrogametocytes and a few microgametocytes were present. An immature macrogametocyte located near the row of nuclei of the epithelial cells of the small intestine is illustrated in Figure 32-I. This stage appears as a large second generation trophozoite, and developed into the mature macrogametocyte (Fig. 33-J), which is much larger with finely granular material.

The microgametocytes measured in the present work, averaged 19.1 by 27.9 microns. Swellengrebel (1914) stated that the microgametocyte measured 35 by 47 microns. He described the development of the microgametes. The chromatin of the microgametocyte became blistered, then worm-like or spindle-form and curved. The microgametes, lying parallel in groups, had two flagella. Although two flagella are characteristic of the microgametes of the species of coccidia, the current investigation did not reveal them since special staining procedures were not employed.

In the present study, the photomicrograph (Fig. 34-K) shows an immature microgametocyte with two large residual masses surrounded by slender nucleated units, the microgametes. As the parasite matured (Fig. 35-L), the residual masses disappeared and the greatly enlarged and distorted epithelial cell was filled with numerous microgametes.

Wenyon (1923) believed that the microgametocyte commenced as one of the larger merozoites. At first it was gregariniform in character, and became irregular in shape until it had a length of about 20 microns. The mature microgametocyte, 30 by 50 microns, contained well over 2000 microgametes. The microgametes, he stated, were 5 microns in length, with pointed anterior and tapered posterior ends.

A small chromatin mass beside the nucleus of the macrogametocyte was labeled the "Nebenkorper" (side body) by Swellengrebel. The light zone surrounding this mass and the nucleus was larger than in the merozoites. The side body disappeared as the gametocyte matured. In the present work, the side body was not observed and the average mature macrogametocyte measured 22.2 by 25.0 microns. He gave the size of the same stage as 45–48 by 48–56 microns and Wenyon gave

the length from 25 to 35 microns. Perhaps the discrepancies in the size of the macrogametocyte as given by the various workers can be partially explained by the difficulty of measuring these ovoidal stages in tissue sections. The parasite may not have been sectioned at its maximum dimensions.

Parthenogenesis of the macrogametocyte was described by Swellengrebel. Wenyon and this present author saw nothing comparable to his account.

The oocysts showing hyaline, unstained walls while still intracellular, averaged 19.3 by 24.5 microns. The photomicrograph of the oocyst (Fig. 36-M) presents the oocyst wall while still in the epithelial cell of the small intestine.

One of the kittens passed unsporulated oocysts in the feces on the seventh day and the other on the eighth day, thus making the prepatent period 7–8 days. Andrews (1926) reported the prepatent period as 5 or 6 days.

On several occasions two asexual stages were found developing in the same epithelial cell. Wenyon (1923) mentioned this phenomenon when he observed that two merozoites had invaded the same cell.

Andrews (1926) artificially infected 8 kittens (wt. 308–750 gm.) with sporulated oocysts of *I. felis*, ranging in number from 100 to 100,000 per animal. He stated that the symptoms of coccidiosis in cats and dogs were the same as in other animals with the host rapidly becoming anemic, emaciated, weak and depressed. In the later stages of the infection, some of the kittens became weakened, prostrated, exhausted and died. The two animals to which he gave 100,000 oocysts of *I. felis* died. He did not state his evidence for the animal's being anemic, except to mention the presence of gross and microscopic blood in the stools. Enteritis, emaciation and frequent deaths were enumerated by Wasielewski (1904) as the symptoms of natural infections of *I. felis* in the cat.

Morgan and Hawkins (1948) listed the pathology produced by the *Isospora* in the cat and dog as hemorrhagic enteritis, frequently with ulceration throughout the small intestine, a thickened mucosa with widespread desquamation of the epithelium, and an extensive amount of connective tissue in the recovered cases and those with repeated infections. This description was well supported with recent investigations of canine coccidiosis; however, their source for the feline description probably emanated from investigations of 1926 or before.

Since symptoms and pathological changes were commonly associated with coccidiosis in other animals, one might expect to find it in kittens. Such was not true, as the author found no evidence of gross or microscopic lesions, edema, hemorrhage, or symptoms attributable to the presence of this organism. The symptoms and deaths recorded by Andrews and Wasielewski might well have been due to feline distemper. Their experimental and naturally infected animals were not vaccinated against feline distemper, since this was not done at that time. Some of the symptoms and pathological conditions stated by them bear a marked similarity to those of this highly contagious, fatal illness of kittens. Perhaps this disease accounted for some of the symptoms and deaths noted by the early investigators.

SUMMARY

The life-cycle of *Isospora felis* in the kitten is presented by means of photomicrographs and drawings of the morphological stages in their relative locations in the tissues. Artificial infections were produced and the animals killed at daily intervals. Tissues, for microscopical examination, were removed at definite locations.

This life-history consists of two asexual cycles and one sexual. The first asexual phase, described for the first time, existed from the second to the fourth days, while the second asexual phase lasted during the fifth and sixth days, and the sexual cycle remained the seventh and eighth days.

Since oocysts were passed on the seventh and eighth days, this presents a prepatent period different from that previously thought to exist.

All parasitic stages of *I. felis* found were intracellular in the surface epithelial cells of the intestine as formerly reported.

Most of the sexual and asexual stages were found in the small intestine, but a few sexual stages were found in the cecum. A few asexual forms were present in the cecum and, for the first time, noted in the large intestine.

The majority of the mature second generation schizonts contained 12, 14, or 16 merozoites.

No pathological conditions or symptoms attributed to this infection were recognized.

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Explanation of Plates

All figures on Plates I and II were drawn free hand to represent the parasite in its relative position to the host's epithelial cells. Scale 1.5 microns equivalent to 1.5 mm.

Plate I

Fig. 1. An immature first generation schizont, 7.0 by 10.0 microns.
Fig. 2. An immature first generation schizont, 7.5 by 9.0 microns.
Fig. 3. A mature first generation schizont, 7.5 by 16.5 microns.
Fig. 4. A mature first generation schizont, 8.0 by 13.5 microns.
Fig. 5. A mature first generation schizont, 6.0 by 13.5 microns.
Fig. 6. A second generation trophozoite, 6.0 by 10.5 microns.
Fig. 7. An immature second generation schizont, 4.5 by 7.5 microns, with two merozoites.
Fig. 8. An immature second generation schizont, 10.5 by 15.0 microns, with four merozoites (only those in one plane were drawn).
Fig. 9. An immature second generation schizont, 9.0 by 10.5 microns, with four merozoites.
Fig. 10. An immature second generation schizont, 13.5 by 18.0 microns, containing four pairs of merozoites, 4.5 by 7.5 microns.
Fig. 11. A second generation schizont, 9.0 by 18.0 microns, containing six merozoites, 3.0 by 10.5–13.5 microns.
Fig. 12. An immature second generation schizont, 12.0 by 15.0 microns, containing four pairs of merozoites, 7.0 by 9.0 microns, in the cecum.

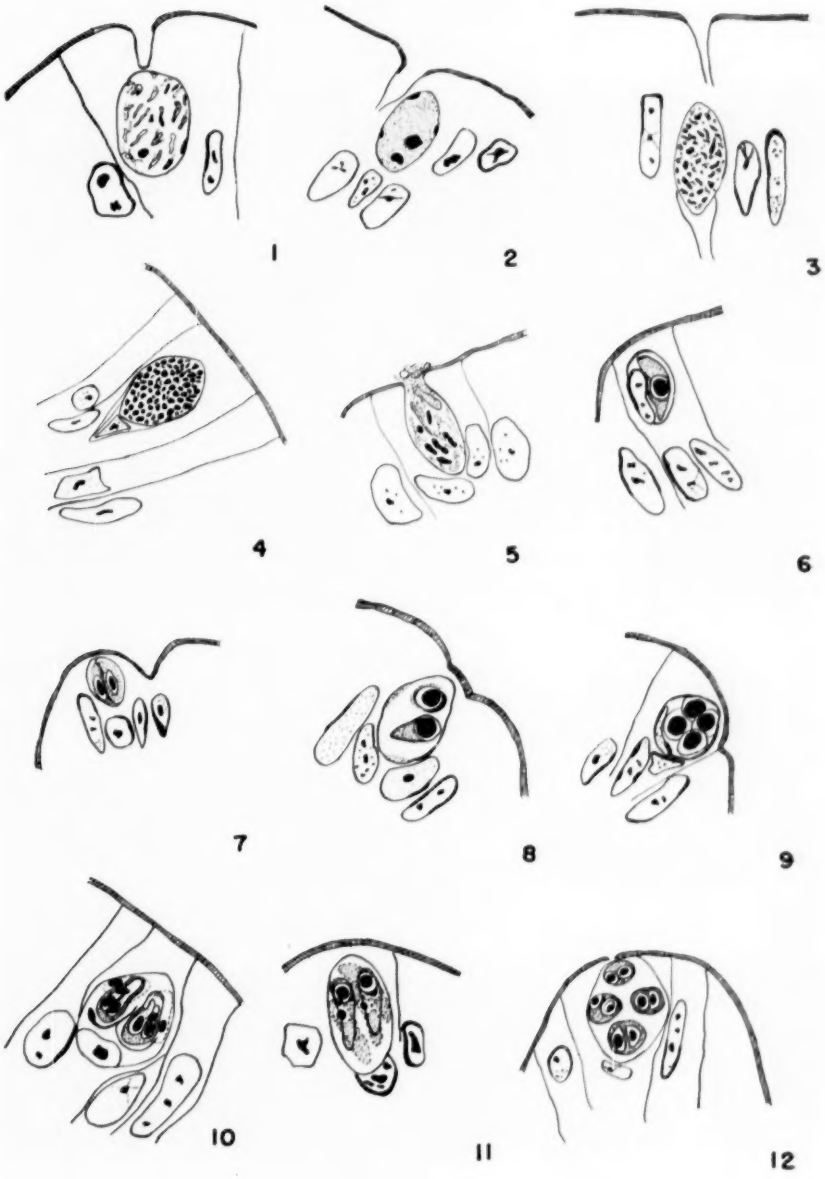
Plate II

Fig. 13. A second generation schizont, 16.5 by 18.0 microns, containing eight merozoites (only those in one plane were drawn).
Fig. 14. A second generation schizont, 9.0 by 34.5 microns, containing 16 merozoites.
Fig. 15. A second generation schizont, 15.0 by 17.0 microns, containing 12 merozoites.
Fig. 16. A second generation schizont, 22.5 by 22.5 microns, containing 20 merozoites.
Fig. 17. A free second generation merozoite, 4.5 by 10.5 microns, in the lumen of the small intestine.
Fig. 18. An immature macrogametocyte, 7.5 by 24.0 microns.
Fig. 19. A mature macrogametocyte, 25.5 by 27.0 microns.
Fig. 20. An immature microgametocyte, 16.5 by 22.5 microns.
Fig. 21. An immature microgametocyte, 27.0 by 27.0 microns.
Fig. 22. An immature microgametocyte, 27.0 by 31.0 microns.
Fig. 23. A mature microgametocyte, 22.5 by 30.0 microns.
Fig. 24. An intracellular oocyst, 23.0 by 29.0 microns.

Plate III

Figures 25 through 36 are unaltered photomicrographs of tissue sections stained with eosin-hematoxylin. They were taken with oil immersion lens at a final magnification of ×1,320.
Fig. 25. First generation schizonts, A and B, of *Isospora felis* in the epithelial cells of the small intestine of a kittten.
Fig. 26. A second generation trophozoite, C, of *I. felis* in an epithelial cell of the small intestine.
Fig. 27. A second generation immature schizont, D, with two merozoites in an epithelial cell of the small intestine.
Fig. 28. A second generation immature schizont, E, with 4 pairs of merozoites in an epithelial cell of the small intestine.
Fig. 29. A second generation immature schizont, F, with 6 merozoites in an epithlial cell of the large intestine.
Fig. 30. A second generation schizont, G, with 14 merozoites in an epithelial cell of the small intestine.
Fig. 31. A second generation schizont, H, with 24 or more merozoites in an epithelial cell of the small intestine.
Fig. 32. An immature macrogametocyte, I, in an epithelial cell of the small intestine.
Fig. 33 A mature macrogametocyte, J, in an epithelial cell of the small intestine.
Fig. 34. An immature microgametocyte, K, in an epithelial cell of the small intestine.
Fig. 35. A mature microgametocyte, L, in an epithelial cell of the small intestine.
Fig. 36. An oocyst, M, in an epithelial cell of the small intestine.

PLATE I



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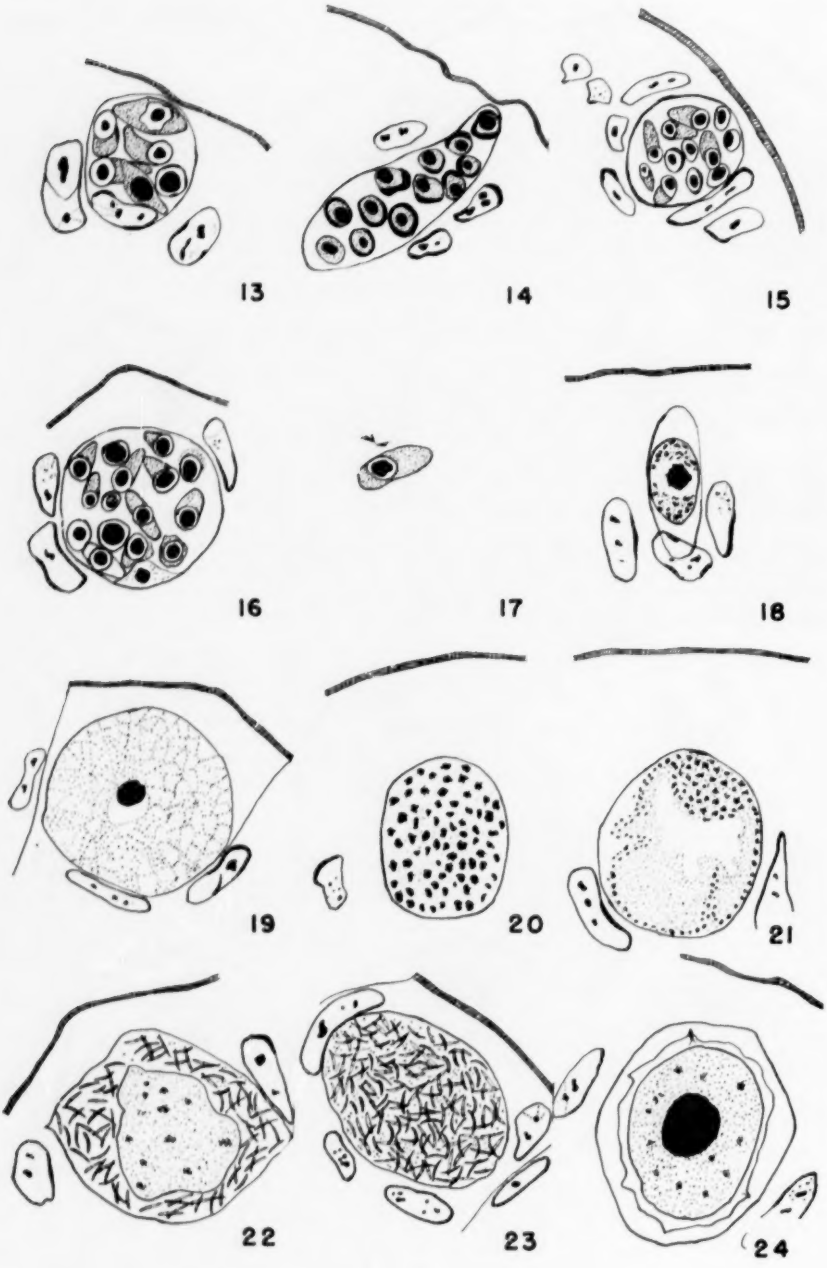
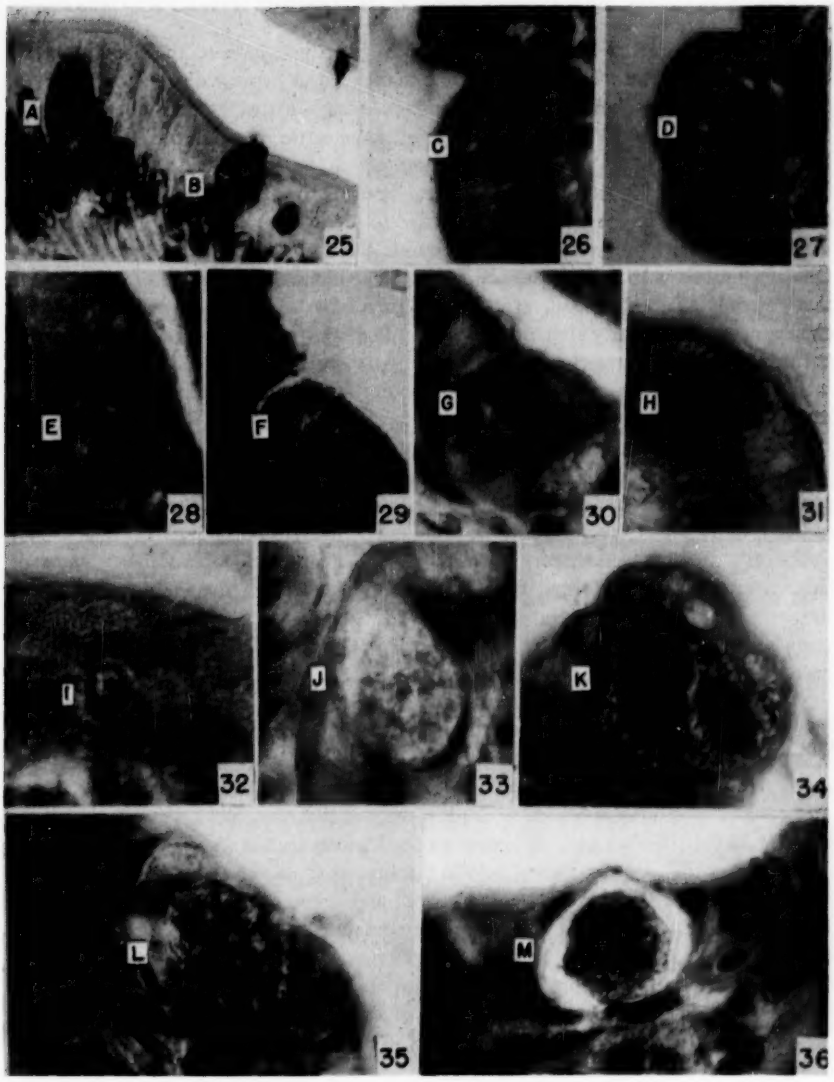


PLATE III



BACTERIA ASSOCIATED WITH WILD AND LABORATORY-REARED HORN FLIES, *SIPHONA IRRITANS* (L.) (DIPTERA: MUSCIDAE)[1]

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The work reported here forms part of an investigation into the life-history of the horn fly, *Siphona irritans* (L.). The main project arose from a need for more effective control of horn-fly populations on range cattle. Horn-fly infestation causes much distress and loss of condition in range cattle in southern Alberta during the summer months, with consequent economic loss (McLintock and Depner, 1954). One of the chief difficulties in the general investigation was that of rearing horn flies continuously in the laboratory in order to study their habits more adequately. These insects have not yet been reared beyond the first generation in the laboratory. One reason seems to be the absence from the artificial environment of some factor that controls ovary development. This in turn may be associated with some deficiency in the laboratory diet of the flies. The well-recognized association under natural conditions between oviposition and cow dung suggests the possibility that internal and environmental bacterial populations supply an ovary-stimulating factor. This report is an account of the bacteria isolated from wild and laboratory-reared flies and from their normal environment.

The bacteria associated with insects have long been the object of study by bacteriologists and entomologists. Interest has centered largely on the possible role of bacteria as pathogens for insects (Steinhaus, 1947, 1949; Wigglesworth, 1950). Practically all insects in nature harbor bacteria of one kind or another. Most of these are present as a normal flora of the alimentary tract, the immature ootheca, the ovaries, the eggs, the integument, or the mycetome (Steinhaus, 1940). A knowledge of the normal flora is a necessary adjunct of any study involving the bacteria of insects.

In comparison with some blood-sucking insects, the bacterial flora of the horn fly has received very little attention. The adult fly is an obligatory blood sucker of cattle, and the larva lives only in fresh cow dung. It is generally believed that the horn fly does not possess hereditary symbiotic microorganisms for the provision of accessory food substances in the adult stage and that they are among those insects that obtain their life's supply of accessory food substances in the larval stage (Wigglesworth, 1929). But Glaser (1923a) found that bacteria are carried over in small numbers from the larval to the pupal and adult stages of the horn fly, and Hammer (1942) noted that horn flies had occasionally been observed drinking the fluid on the surface of freshly passed cattle dung. These observations suggested that the flies might obtain necessary microorganisms from their environment.

In its parasitic habit the adult horn fly is sometimes likened to the lice and hippoboscids (Glaser, 1923b; Neveu-Lemaire, 1938) because it remains almost con-

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stantly on its host. It leaves the host only to deposit its eggs on freshly passed dung, an operation that requires ten minutes at the most (McLintock and Depner, 1954). Consequently the environment of the fly is spatially limited since it includes only the hide of the host and the surface of the fresh dung.

MATERIALS AND METHODS

Wild flies were collected from cattle at the Lethbridge Stockyards, and occasionally from herds at various localities in southern Alberta. The flies were obtained by sweeping the backs or bellies of the cattle with a net. Netted flies were transferred, without anaesthesia, to Erlenmeyer flasks which were then plugged with cotton. Early in the investigation the wild flies were caged on their arrival at the Lethbridge laboratory and shipped alive to Edmonton on the day on which they were collected. Later, when eggs were required for rearing, the wild flies were first placed over fresh cow dung in battery jars for 24 hours. No food was supplied to the flies and, during this time, their eggs were deposited on the dung and around the walls of the jars. At the end of the 24 hours the surviving flies were shipped to Edmonton.

The larvae were reared in fresh cow dung in battery jars. If the dung appeared to be very wet, a two-inch layer of sand was placed in the bottom of the jar before the dung was added. Eggs that were laid on the dung were left undisturbed to hatch, and the larvae were allowed to complete their development in the jars. Eggs that were laid in the Erlenmeyer flasks were washed out with water and transferred to filter paper in a Buchner funnel. After the excess water had been drawn off by suction, the eggs were brushed onto the surface of fresh dung in battery jars.

The battery jars were kept in cabinets at 25°C. At this temperature the eggs hatched in less than 24 hours and the larvae completed their development in about six days. When sand was used in the battery jars the puparia were found in the sand. The sand was added to water and stirred, so that the puparia floated to the surface and could be removed with a small strainer. When no sand was used, the puparia were found in the drier parts of the dung, just under the crust. In this case the crust was removed and the slightly moist and caked dung underneath the crust was broken into a Waring blendor. Water was then added and the mixture was agitated slowly. When the dung had settled, the puparia could be removed with the strainer. Also, washing the puparia in water was necessary to remove parasitic mites.

The puparia were distributed in Petri plate lids that were placed in the bottom of the cage described by McLintock (1952) and the flies were allowed to emerge there. At 25°C. emergence began about 5 days after formation of the puparia. The females began to emerge first, and, in order to obtain a balanced mixed population, flies were not removed from the emergence cage until a day or two had elapsed.

For shipment to Edmonton, flies were transferred by means of an aspirator to cylindrical wire cages. These cages were 5½ inches high and 4½ inches in diameter, the wall being of 20-mesh galvanized iron screening. One end of the cylinder was covered with light cotton and the other end was closed with a light cotton cloth sleeve. Each cage received up to 1,000 flies. A cage containing the flies was then placed in a cardboard carton 5 inches square and 6½ inches high.

The carton was lined with heavy wax paper. A pad of moist cellucotton was placed on the bottom of the carton and the cage placed on top of the pad. The carton was then closed and sealed with kraft tape. The cartons were shipped the 340 miles to Edmonton by railway or air express; only an occasional fly was dead on arrival. The cages were sterilized in an autoclave before each shipment to prevent contamination from one collection to another.

Isolation of Microorganisms from S. irritans.

On arrival in Edmonton living flies were chosen at random for examination and the remainder placed in a deep-freeze. Each batch examined contained one to 50 flies. In all experiments the flies were killed by applying the point of a red hot needle to the head of each insect. Thus the body of the fly remained intact and undamaged.

Seven batches (72) wild flies were examined for external microorganisms. For the isolation and culture of external microorganisms, an intact, killed fly was placed in nutrient broth for 24 hours and incubated at 26° or 37°C. The resulting growth, as shown by clouding of the broth, was inoculated on nutrient, enriched and selective media. The inoculum was distributed evenly, with a sterile glass spreader, over the surface of each medium in Petri plates. The media used in isolating microorganisms from each specimen were: one nutrient agar plate incubated at 26°C. for 24 hours; two blood agar plates incubated at 37°C. for 24 hours, one plate aerobically and the other anaerobically; one MacConkey's agar plate incubated at 37°C. for 24 hours; and one Littman's agar plate held at room temperature for one month (this medium was employed for the isolation of yeasts, molds, and fungi). Individual colonies were picked from these plates to nutrient agar slants or Loeffler's serum slants for storage and later identification. A direct smear of each kind of microorganism was made at the time individual colonies were picked. Smears were prepared by heat fixation and stained by Gram's method.

Before a fly could be examined for internal microorganisms it was necesary to eliminate contamination from the external surfaces of the fly. The most satisfactory technique was to immerse the fly in a 1 : 500 alcoholic solution of bichloride of mercury for 20 to 30 minutes. The disinfectant was then removed from the fly by washing it in sterile physiological saline for two 10-minute periods. In order to determine whether the fly had been sterilized externally, it was incubated at 37°C. for 96 hours in nutrient broth. Flies showing external growth after treatment with disinfectant were discarded. A total of 398 flies, slightly over 70 percent, treated with disinfectant were suitable for culture of internal microorganisms. If the broth in which the fly had been suspended showed no cloudiness, that is, no bacterial growth, the fly was crushed against the side of the tube with a sterile glass rod. The crushed flies suspended in broth, individually or in batches, were then incubated and the bacteria that grew were isolated in pure culture. No appreciable qualitative differences were found in the results of culture of the internal organs of the horn flies and no evidence was obtained of any interference with the viability of internal microorganisms cultured after the use of disinfectants or following the prolonged incubation carried out to ensure freedom from surface contaminants. A smear of each pure culture was made as each colonial type was picked for stock culture. The number of flies examined in this way was 243.

Dissection of *S. irritans* was necessary for microbiological examination of the gut and interior of the fly. The exterior of the fly was disinfected as described above and aseptic technique was employed during dissection. After removal from the saline rinse, the fly was dried externally so that it could be embedded in paraffin wax. No one method for drying seemed best, but immersion in ether or alcohol followed by passage through a flame offered the most rapid method when a small number of flies were being prepared for dissection. When large numbers were dissected the flies were placed on sterile blotting paper in Petri plates. Drying by this method took approximately 45 minutes, during which it was necessary to replace the blotting paper two or three times as it became moist. When each fly was dry it was embedded, dorsal surface down, with one half of the body above the surface of the wax in a Petri plate. Before embedding, the paraffin was heated for 15 minutes to destroy contaminating microorganisms. After the fly was embedded and the paraffin hardened, an amount of Ringer's frog heart solution sufficient to cover the fly was poured into the Petri plate. A microscope, a good source of light (100-watt lamp), and dissecting instruments were employed for dissection. The instruments were sharply pointed forceps, a pair of fine scissors, scalpels with detachable blades, and needles. A dissecting needle bent in the middle at a 90° angle and fixed in a glass rod handle was most useful because of its manoeuverability. The abdomen of the fly was opened on the ventral surface by making an incision from the constriction of the thorax to the anus. After the abdomen was opened, two dissecting needles were used to expose the gut by teasing away the other viscera. The anterior intestinal tract was removed from the thorax by holding the ileum with a pair of forceps and pulling the gut back. An incision through the constriction of the rectum was made to detach the gut from the fly. The guts, in batches of one to 25, depending on the material available at the time, were then transferred to nutrient broth and crushed for culture. A total of 106 flies were examined by this procedure.

Direct fecal smears from living flies were made by squeezing the abdomen of each fly with forceps, smearing the feces on slides, and staining with Gram's stain. Forty-one smears were prepared in this way in 1951 and 72 in 1952.

The technique used for whole flies was employed in examining eggs from wild females, first-instar and third-instar larvae, and pupae for internal organisms. No second-instar larvae were examined.

Isolation of Microorganisms from the Environment.

Four perianal swabs from cattle and 12 fecal specimens collected at Lethbridge were examined bacteriologically. For the isolation of microorganisms, each specimen was inoculated on solid nutrient, enriched and selective media, the same procedure being followed as for the isolations from the external surfaces of the flies.

Isolation of microorganisms present on the skin of cattle was accomplished in the same way. The flies, before darting to the dung, are frequently seen resting on the pastern, hock, and pin bones of the host. Swabs of these three areas were taken from each of six cattle at Lethbridge and shipped to Edmonton on nutrient agar slants and in special anaerobic transport medium (Moffet, Young, and Stuart, 1948).

Classification of Bacteria.

The criteria for bacterial identification were based on the recommendations of Bergey's *Manual of Determinative Bacteriology,* sixth edition, 1948, and were confined to morphological and biochemical characters. Only characters were studied that could be used to identify, with reasonable accuracy, the organism as far as genus or species. Time and the facilities available did not permit a quantitative study of the bacterial populations found although it was obvious, during the work, that certain species were encountered more frequently than others.

Results

Microorganisms Isolated and Identified.

Table 1 shows the bacteria found associated with wild and laboratory-reared specimens of *S. irritans.*

Table 1.—*Bacteria associated with wild and laboratory-reared specimens of* S. irritans

Microorganism		Environment		Wild adults			Laboratory-reared specimens				
Genus	Species	Feces of cattle	Skin of cattle	Exterior	Interior	Intestinal tract	Eggs†	First-instar larvae	Third-instar larvae	Pupae	Crushed unfed adults
Achromobacter		•+	+	+	+	+	−	+	−	−	−
Aerobacter	aerogenes	−	+	−	−	−	−	−	−	−	+
	cloacae	•−	−	−	−	−	−	−	−	−	+
Alcaligenes		+	+	+	−	+	−	−	−	−	−
Bacillus		•+	+	+	+	+	+	+	−	−	−
	subtilis	−	−	−	+	−	−	−	−	−	−
	subtilis var. niger	−	−	−	−	+	−	−	−	−	−
Escherichia	coli	+	+	+	+	−	−	+	−	−	+
	freundii	−	+	−	−	−	−	−	+	+	−
	intermedium	+	+	+	+	−	−	−	+	−	+
Flavobacterium		+	+	−	−	−	−	−	−	−	−
Micrococcus	aurantiacus	•+	+	−	−	−	−	−	−	−	−
	candidus	•+	+	+	−	+	−	−	−	−	+
	caseolyticus	•+	−	+	−	−	−	−	−	−	−
	epidermidis	•−	+	+	+	+	−	−	−	−	+
	flavus	+	+	−	−	+	−	−	−	−	−
Proteus		•−	+	−	−	−	−	−	−	−	−
	rettgeri	•−	−	−	+	−	−	−	−	−	−
Unidentified aerobic rods	small and medium size, non-sporing Gram positive	+	−	+	−	−	+	−	−	−	−
	medium Gram neg.	+	−	−	−	−	−	−	−	−	−
	large, non-sporing Gram positive	−	+	−	−	+	−	−	−	−	−
Unidentified anaerobic rods	small, Gram negative	−	+	−	−	−	−	−	−	−	−
	medium, non-sporing Gram positive	−	−	−	−	−	−	+	−	−	−
	large, non-sporing Gram positive	+	−	−	−	+	−	−	−	−	−

* Organism present; − organism not found on examination; • also isolated from laboratory-reared flies fed *M. flavus;* † the ova were deposited in the laboratory by wild females.

Nine batches comprising 117 crushed wild flies and four batches comprising 18 unfed laboratory-reared flies contained internal organisms. The digestive tracts of 72 wild flies (28 batches) contained organisms. Two batches comprising 33 eggs, 42 individual first-instar larvae, and two individual third-instar larvae, and one batch

comprising 50 pupae contained internal organisms. No bacteria were isolated from six batches comprising 23 eggs and three separate first-instar larvae, or from two batches comprising six third-instar larvae.

Organisms isolated from laboratory-reared flies that were fed aqueous suspensions of *Micrococcus flavus* are not included in the table. This organism was previously isolated from a batch of ten wild flies that were externally sterilized and crushed. *M. flavus* was not recovered from eight batches comprising 49 flies that had been fed aqueous suspensions of this organism, but 14 other bacterial organisms were obtained. Nine of these were obtained from other scources during the investigation and are indicated in Table 1; the other five were *B. cereus* var. *mycoides; Streptococcus;* one unidentified, aerobic, large, non-sporing, Gram-positive rod; and two unidentified, anaerobic, Gram-positive rods.

Of 20 batches comprising 243 externally sterilized and crushed wild flies, internal organisms were found in nine batches comprising 117 flies. In order of frequency of isolation, the organisms were *Esch. coli,* from four batches (60 flies); *P. rettgeri,* from four batches (100 flies); *M. epidermidis,* from three batches (51 flies); *B. subtilis,* from three batches (35 flies); *Esch. intermedium,* from one batch (25 flies); a species of *Achromobacter,* from one fly; and a species of *Bacillus,* from one fly. These organisms represent bacteria of the internal organs and body cavity.

From the dissected intestinal tracts, *M. epidermidis* was isolated from 11 batches (23 flies); a species of *Achromobacter,* from ten batches (26 flies); *M. candidus,* from three batches (seven flies); *M. flavus,* from two batches (six flies); a species of *Alcaligenes,* from two batches (ten flies); a species of *Bacillus,* from two batches (21 flies); *B. subtilis* var. *niger,* from one batch (two flies); and an unidentified organism from one fly. No organisms were isolated from the intestinal tracts in 29 batches (34 flies). In many instances more than one type of organism was isolated from the same batch of flies and, in some instances, from the same fly.

Of the 41 direct smears of horn-fly feces prepared in 1951, 37 showed the presence of either Gram-positive cocci or Gram-negative bacilli or both in large numbers; no organisms were seen in four smears. Of the 72 smears prepared in 1952, 26 showed the presence of Gram-positive cocci alone; one showed Gram-positive cocci and Gram-negative rods present together; no organisms could be seen in 45 smears.

In 52 samples cultured individually from the same batch of horn flies, *M. epidermidis* was isolated from ten, and from two an unidentified Gram-negative rod was grown. No growth occurred in the remaining 40 samples.

Although a search was made in histological sections of horn flies for possible intracellular symbionts, none was observed.

Organisms of the genera *Aspergillus, Cladosporium, Penicillium, Oidium, Geotrichum, Trichothecium,* and *Pullalaria,* an organism in the order PHOMALES, and one with the morphology of an actinomycete were isolated from one or more of the sources under investigation. Since these organisms appeared neither consistently nor in large numbers in cultures from the horn fly and its environment, they were not studied further.

DISCUSSION

The results thus far agree with the contention of Glaser (1923a) that very few bacteria are carried over from the immature stages of the horn fly to the adult. In this study only seven kinds of bacteria were found in the eggs and immature stages, and none of these bacteria was present in all the stages of the fly that were examined. Hence it appears that the horn fly does not contain a constant bacterial flora as represented by those bacteria that can be cultured by standard bacteriological methods. Smears of fly feces and intestinal tracts indicated that it is unlikely that many additional unculturable bacteria are present. On the other hand, bacteria frequently occurred inside adult horn flies (of 77 batches [349 flies], 37 batches [189 flies] contained internal bacteria).

Evidence that the organisms found in the intestinal tract may tend to keep each other's numbers in check was obtained in tests for antagonistic action. In these tests any evidence of growth inhibition was checked by a repeat test. *M. epidermidis* and *M. flavus* exerted a pronounced inhibitory effect on *Bacillus brevis;* on two unidentified species of *Bacillus;* on an unidentified anaerobic Gram-positive rod; on two unidentified, large, aerobic Gram-positive rods; and on one large, aerobic Gram-negative rod occurring in the intestinal tract of the fly. One strain in each of the genera *Achromobacter* and *Alcaligenes* exerted variable inhibitory action on the same organisms. One strain of the genus *Achromobacter* had an inhibitory action on another strain of the same genus and a lesser inhibitory action on *M. epidermidis* and *M. flavus*. Another strain of the genus *Achromobacter* and one strain of the genus *Alcaligenes* had a slight inhibitory action on two other strains of the genus *Achromobacter*. *M. epidermidis* had a pronounced inhibitory effect on one strain of the genus *Achromobacter*. This antagonism probably has an effect on the relative numbers of individual strains present at any one time.

In appraising the results obtained in this study the limitations of the methods used must be kept in mind. The microbial life in the liquid of cow feces is abundant as seen under the microscope, but very few of the organisms seen can be cultured. MacNeal, Latzer, and Kerr (1909), in their extensive work on the fecal flora of man, could culture on the average only one out of each 3,000 cells seen with the microscope. Hastings (1948) states that similiar results have been obtained with the feces of cattle and other animals. Satisfactory laboratory media are not yet available for many bacteria and the bacteria isolated in the present study were only those that grew on the common laboratory media. In this study 15 species of bacteria were isolated from the skins of the cattle and only 13 from their feces. On the other hand, the examination of smears of the horn-fly feces revealed no greater variety of morphological bacterial types than were isolated from cultures of the dissected intestines of the fly.

One would expect the larvae, as inhabitants of the dung, to have a high bacterial content. This inference is supported by the isolation of bacteria from 42 out of 45 batches of first-instar larvae. From third-instar larvae, however, only two species of bacteria were isolated (Table 1). These two organisms were both obtained from a single larva out of a total of eight that were about to form the puparium. The majority of insect larvae stop feeding just before they molt; hence, most of the microorganisms present in the feeding larva might be eliminated or digested at this time, and only a few persist in the pupa. Only one microorganism was

isolated from one batch of 50 puparia. This organism, *Esch. freundii,* was also present in the larvae and on the skin of cattle but was not found in the intestines of wild flies. Since a number of microorganisms considerably in excess of those found in pupae were found in the intestines of adult wild flies, one can only conclude that the majority of organisms in the adult insects are obtained from their environment.

Samples of feces and skin swabs were taken from one herd and the samples of flies from other herds, most of which were pasturing at a considerable distance from the first. This was considered an advantage because the organisms present generally in the flies were of more interest than local floras. But the fact that no bacteria were isolated from the crushed bodies of 126 flies (11 batches) suggests that bacteria are not consistently present in the flies. Five identified and one undentified bacteria were found in the insects but not in the environment. Two of the identified organisms (*Aero. cloacae* and *B. cereus*) and the unidentified organism were isolated only from laboratory-reared specimens, suggesting that these organisms were picked up by the insects in the laboratory. Two of the others (*B. subtilis* and *B. subtilis* var. *niger*) were isolated only from the wild flies and are probably representative of local floras. The fourth (*P. rettgeri*) was isolated from both laboratory-reared and wild flies.

We have listed the organisms isolated from the crushed bodies of the flies as internal, but this is on a purely anatomical basis. Some of these organisms may have been present in the fore gut, the hind gut, or the tracheae, which are morphologically external and more or less continuous with the exterior.

If the flies may be considered to possess a normal bacterial flora, only those organisms found in both cow dung and the interior of the wild flies (recalling that the dung and the flies came from different herds), and those organisms found inside both the wild and the unfed laboratory-reared flies, can be considered as members of the normal flora. Those found both in the dung and inside the wild flies were: *Achromobacter, Alcaligenes, Bacillus, Esch. coli, Esch. intermedium, M. candidus, M. flavus,* and one unidentified organism. Those found inside both wild and unfed laboratory-reared flies were: *Esch. coli, Esch. intermedium, M. candidus,* and *M. epidermidis.* The identified bacteria are all normal associates of cattle (*vide* Bergey). In the variety of organisms found, there was nothing to suggest that there is a bacterial flora specific to *S. irritans,* and one can conclude that the bacteria found in the horn fly are the bacteria of its immediate environment. Inasmuch as certain bacteria are normal associates of cattle, they can also be considered as normal associates of horn flies.

M. flavus was not recovered from the 49 flies that were fed this organism. This indicates that it had been either eliminated with feces or digested; but other organisms were present in these flies so that the possibility of elimination of *M. flavus* by antagonistic bacterial action must be considered. At least two organisms (*B. subtilis,* and *Esch. coli*) isolated from the interior of wild horn flies, and one (*B. cereus*) from the interior of fed laboratory-reared flies, are known to be capable of producing antibiotic substances (Baron, 1950).

SUMMARY

Bacteria were isolated from the environment of wild horn flies; from the exterior, interior, and intestinal tracts of wild flies; from eggs, larvae, and pupae;

and from the interior of unfed laboratory-reared flies. Thirteen organisms representing five genera of bacteria were identified to species, six to genus, and 12 remained unidentified. No organisms were isolated from the interiors of 126 wild flies (11 batches) out of 243 flies (20 batches).

The paucity of isolations from the larvae and pupae support Glaser's belief that very few bacteria are transmitted from larva to adult. It seems that most of the bacteria in the adults are obtained from their environment.

It is concluded that the normal internal bacteria of adult horn flies are normal associates of cattle and include *Esch. coli, Esch. intermedium, M. candidus, M. flavus, M. epidermidis,* and unidentified species of *Achromobacter, Alcaligenes,* and *Bacillus.*

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A SUMMARY OF THE GENERA OF MYOBIIDAE (ACARINA)

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There have been several recent papers dealing with the mites of the family MYOBIIDAE but there is sufficient need for a brief discussion of the genera of this group. The current status of some genera and allocation of the species have not been made with a consideration of their host relationships, there has been some confusion of the various life stages, and consequently the classification is now somewhat misleading. Ewing (1938), in a paper on the North American species, was the first to separate different groups from *Myobia* and place them in other genera. The most lengthy recent review of the MYOBIIDAE is that of Radford (1948). This paper was published in 16 installments over a six year period, but the generic names date from the first (1948) installment. Because of the delay in publication of Radford's paper, it was impossible for Radford (1948 and 1954) on the one hand, and Lawrence (1951 and 1954), Jameson (1949), and Jameson and Chow (1952) on the other, to examine the results of each other's efforts. These papers differ so that a reconsideration of the genera is now required. It is the purpose of this brief account to establish a basis of departure for future students of these mites.

Myobiid mites are parasitic on mammals of the orders INSECTIVORA, CHIROPTERA, and RODENTIA. An interesting new genus is from an opossum of the order MARSUPIALIA.

Archemyobia, new genus

Leg I (Fig. 1, A and D) rather long for the family, of five segments; with two conspicuous and strongly curved tarsal claws, quite unlike leg I of the adults of any known myobiid genus. Second and third segments of leg I with hook-like lateral projections. The two tarsal claws on leg II subequal; paired tarsal claws on legs III and IV unequal, the posterior claw on each leg about one-fourth as large as the anterior claw (Fig. 1, C). Body about two and one-half times as long as broad. Male unknown. Genotype *Archemyobia inexpectatus*, new species.

Archemyobia inexpectatus, new species

Female

Dorsum (Fig. 1, C): Laterals I–III and submedians I and II all moderately expanded and striated, the striations running toward the midline of each seta. Dorsal setae acuminate and unbarbed. Submedians III–VI smaller, scarcely expanded or striated. Two pairs of circumanal setae, similar to submedians III–VI. A pair of rather heavy, anteriorly projecting setae on the third and fourth segments of legs III and IV.

Venter (Fig. 1, B): With three converging rows of setae as shown, slender, unstriated and unbarbed. Two more pairs of short, simple setae near the caudal margin.

Legs: Leg I (Fig. 1, A and D) with a striated seta on the second segment and two striated setae on the third segment, dorsally. The two large terminal claws are equal. Legs II–IV (Fig. 1, B and C) with setae and claws as illustrated; claws on legs III–IV markedly unequal, the anterior claw three or four times larger than the posterior claw.

The male is unknown.

Host: Opossum, *Didelphis virginiana* Kerr.

Type locality: Maryland, Patuxent Wildlife Research Refuge; 14 January 1950; coll. E. Ediger.

Types: Holotype female deposited with the U. S. National Museum, slide number 2204.

Remarks: The most primitive genus is *Archemyobia* from marsupials, and this genus is logically allied to *Neomyobia* (*e.g., rollinati, chiropteralis,* and *caudata*) from bats.

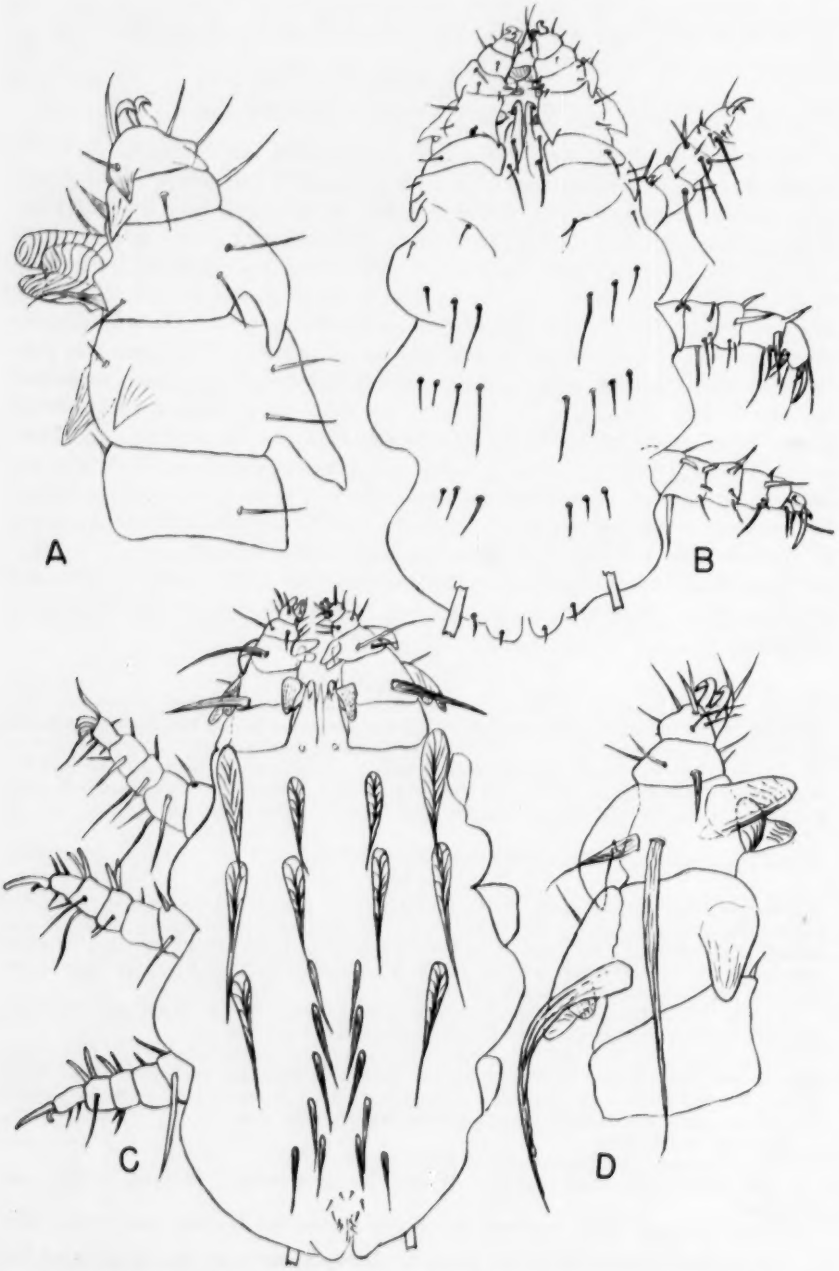

FIG. 1. *Archemyobia inexpectatus.* A, leg I, ventral; B, ventral; C, dorsum; D, leg I, dorsal.

Although both are very primitive orders, the differences between the CHIROPTERA and MARSUPIALIA are profound; there are however several similarities between their myobiid parasites. The likelihood of a relatively recent exchange of myobiid mites seems most remote. The similarities between *A. inexpectatus* and *Neomyobia* are readily apparent in the body shape, character of dorsal setae, the claws, and especially leg I (cf. Fig. 2, B).

In order to discuss the myobiid mites from bats, it is necessary to redefine the genera from this order; there are at present more names than generic entities and a regrouping of the species from bats will clarify their relationships. The interesting and excellent paper by Grant (1942) seems to have been overlooked. This account traces the developmental stages of *Myobia musculi;* and, in most respects, the descriptions of the larvae, nymphs, and adults closely parallel stages in the allied genus *Radfordia*. Grant differentiates between the nymphs and adults. Some synonyms pointed out in the following discussion resulted from a previous confusion of nymphs with females.

Account of the Genera

Archemyobia, new genus

Characters listed above. Known only from the opossum (*Didelphis virginiana*) of the Western Hemisphere. Quite likely this genus will be found on other genera of marsupials (*e.g., Chironectes* and *Metachirus*) of the New World, and possibly even on marsupials of the Australian Region. Only the genotype, *A. inexpectatus*, n. sp., in known.

Neomyobia Radford, 1948

Leg I of four or five segments, with or without terminal claws (Figs. A and B). Legs II–IV each with a pair of claws. Body elongate, and some dorsal setae expanded and markedly striated. Penis slender, sinuate, not coiled or doubled. From bats. Genotype, *Neomyobia rollinati* (Poppe, 1908). Also included in this genus are *N. chiropteralis* (Michael, 1884), *N. caudata* (Banks, 1909), *N. mystacinalis* (Radford, 1935), *N. magna* (Radford, 1934), *N. inaequalis* (Ewing, 1938), *N. jacksoni* (Radford, 1940), *N. plecotia* (Radford, 1938), *N. clara* (Womersley, 1941), *N. pantopus* (Poppe and Trouessart, 1895), *N. poppei* (Trouessart, 1895), *N. unciger* (Lawrence, 1951), *N. natalensis* (Lawrence, 1951), *N. capensis* (Meillon and Lavoipierre, 1944), and *N. africana* (Lawrence, 1951).

The genus *Neomyobia* is based upon *Myobia rollinati* Poppe, 1908. The generic characters of *Myobia chiropteralis* Michael, 1884, genotype of *Foliomyobia* Radford, 1948, and of *Myobia magna* Radford, 1934, genotype of *Binuncus* Radford, 1954, are the same as those of *N. rollinati;* and accordingly *Foliomyobia* and *Binuncus* must be synonymized with *Neomyobia*.

Acanthophthirius Perkins, 1925

Leg I of four segments, without terminal claws (Fig. 2, C). The first segment of Leg I (at least in one species) with a stout, striated projection (seta) dorsally, on the inner side, similar to that of *Calcarmyobia*, but smaller. Legs II with a pair of claws, unequal in size (the posterior being slender). Legs III and IV each with a single claw. Body long and slender; some dorsal setae expanded and with marked striations. From bats. Genotype *A. etheldredae* Perkins, 1925. Also in *Acanthophthirius* are *A. barnleyi* (Radford, 1951), *A. fortuitous* (Radford, 1952), and *A. longa* (Ewing, 1938) (syn. *Ewingana bispinosa* Radford, 1948).

This genus, originally described as a genus of ANOPLURA, proved to be a myobiid mite, and for many years has been placed as a synonym of *Myobia*. The genotype, *Acanthophthirius etheldredae* Perkins, was later redescribed by Radford (1941). This mite is typical of many common forms of bats, and is quite unlike *Myobia sens. str.* The body form, dorsal setae, and legs resemble those of *N. chiropteralis, N. caudata, A. longa, A. fortuitous, N. mystacinalis, N. rollinati* and some others from bats.

Ewingana Radford, 1948 is characterized by the same features seen in *Acan-*

thophthirius. In his original description, Perkins (1925) did not mention the number of claws; Perkins believed that his specimen was a louse, and the resulting description is unusual in several respects. A recent letter from Dr. G. Owen Evans states that on Perkins's type, *Acanthophthirius elthedredae,* "there are two claws,

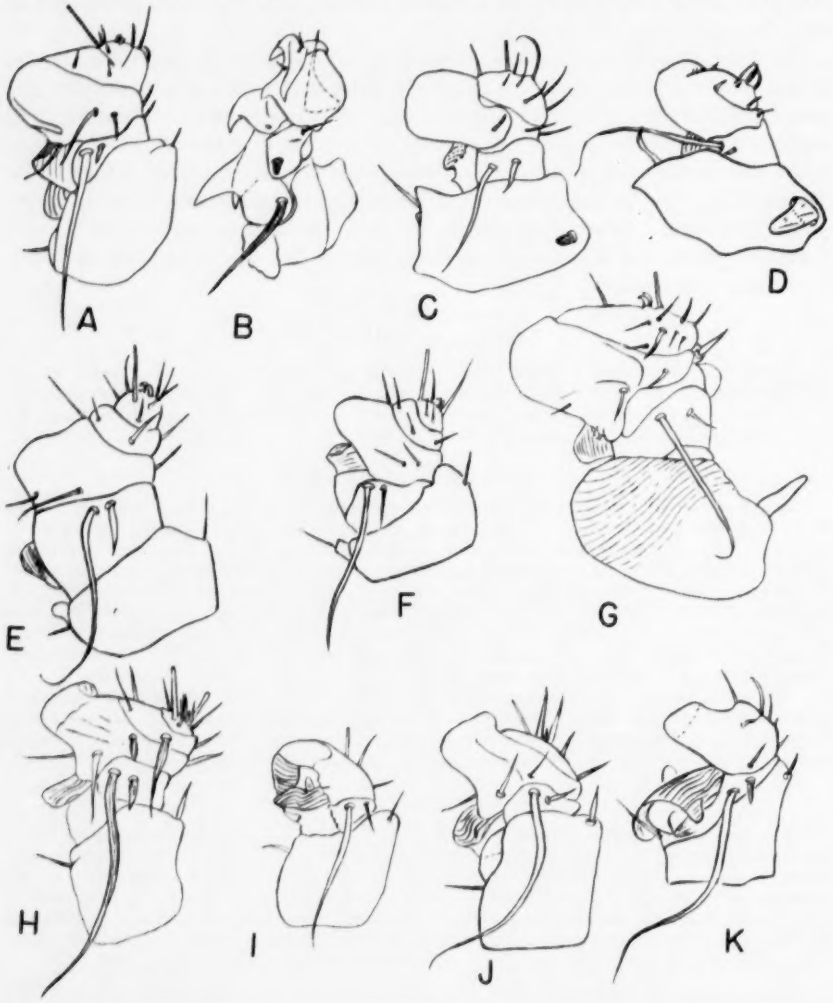

FIG. 2. Leg I of genera of MYOBIIDAE; all adult except B. A, *Neomyobia rollinati;* B, *N. caudata,* nymph; C, *Acanthophthirius longa;* D, *Calcarmyobia rhinolophia;* E, *Protomyobia brevisetosa;* F, *Pteracarus chalinolobus;* G, *Eutalpacarus peltatus;* H, *Eadica brevihamata;* I, *Amorphacarus elongatus;* J, *Blarinobia simplex;* K, *Radfordia ensifera.*

one heavy and one slender on Leg II, and that there is one claw only on each of Leg III and Leg IV." In the absence of any other distinguishing characters of *Ewingana,* it becomes a synonym of *Acanthophthirius.*

An examination of Ewing's type material of *Myobia longa* and of additional specimens from the type host, *Tadarida mexicana* (Saussure) reveals that leg II bears a pair of claws; there is only a single claw on legs III and IV. These features, and the setal pattern, are just as in Radford's description and illustration of *Ewingana bispinosa*. The host of *E. bispinosa* is a species of *Nyctinomus*, a synonym of the widely distributed bat genus *Tadarida*. There is really no basis for believing that *E. bispinosa* is distinct from *A. longa*.

Calcarmyobia Radford, 1948

Leg I of four segments. The basal segment expanded mesally, with a stout, striated, projection (apparently a seta) dorsally (Fig. 2, D). No terminal claws evident. Legs II–IV each with a pair of claws. Body elongate, some dorsal setae expanded and markedly striated. Penis slender, sinuate, not coiled or doubled. From bats, *Rhinolophus lobatus* Peters and *Miniopterus natalensis* (A. Smith). Only one species known, *C. rhinolophia* (Radford) (syn. *Myobia miniopterus* Womersley).

Pteracarus Jameson and Chow, 1952

Leg I (Fig. 2, F) of five segments, with two terminal claws (very similar to *Protomyobia*). Legs II–IV each with a pair of strongly curved, stout claws. Body setae slender and some barbed. Body broad and short. Penis slender, coiled near tip. From bats. The genotype is *Pteracarus chalinolobus* (Womersley, 1941) (syn. *Myobia chalinolobus* Womersley, 1941); also included are *P. tenax* Jameson and Chow, 1952, *P. pipistrellia* (Radford, 1938), *P. minutus* (Radford, 1940), *P. ceylonicus* (Radford, 1951), and *P. pusillus* (Lawrence, 1951).

Protomyobia Ewing, 1938

Leg I (Fig. 2, E) of five segments, with two terminal claws. Leg II with a pair of unequal claws, the anterior being heavier and with a small subterminal notch. Legs III and IV each with a single claw. Body setae slender and some barbed. Body broad and short. Penis rather stout, sinuate. From shrews (Soricidae). Genotype is *Protomyobia claparedei* (Poppe, 1896) (syn. *Myobia claparedei* Poppe, 1896); also included are *P. brevisetosa* Jameson, 1948 and *P. phillipsi* (Turk, 1945). Radford (1954: 239) placed *phillipsi* in *Myobia*, stating that the host (*Neomys fodiens*) is a vole. Actually *Neomys* is a shrew, and *P. phillipsi* appears to be closely allied to *P. claparedei*.

Eadica Jameson, 1948

Leg I (Fig. 2, H) of five segments, with two terminal claws. Leg II with a pair of subequal claws. Legs III–IV each with two terminal claws, the posterior claw being rather slender. Submedians I inflated and striated; other setae slender, non-striated, non-barbed. Body moderately broad, between *Neomyobia* and *Protomyobia*. From insectivores (Soricoidea). Genotype is *Eadica condylurae* Jameson, 1949. Other species assigned to this genus are *E. brevihamata* (Haller, 1882), *E. blairi* (Radford, 1936), *E. crocidurae* (Lawrence, 1951), *E. ingens* (Vitzthum, 1914), and *E. michaeli* (Poppe, 1896).

Eutalpacarus Jameson, 1949

Leg I (Fig. 2, G) of four segments (the fourth and fifth segments apparently fused), with two terminal claws. Legs II–IV each with a pair of terminal claws, the posterior claw being considerably more slender than the anterior claw. Body setae slender and non-striated or weakly striated; some setae with bulbous inflations basally (in the genotype). Body shape moderately wide, as in *Eadica*. Penis stout and curved. From moles, Talpidae. Genotype and only described species is *E. peltatus* Jameson, 1949, from *Neurotrichus gibbsii* (Baird) of western North America. An unnamed species has been collected in Japan from the closely related *Urotrichus talpoides* Temminck.

Amorphacarus Ewing, 1938

Leg I (Fig. 2, I) apparently of three segments, the fourth sometimes concealed beneath the third; one pair of terminal claws; first pair of legs markedly unequal in size. Leg II with a pair of claws; legs III and IV each with a single claw. Dorsal setae slightly inflated, non-striated. Body shape rather slender. Penis very long and doubly coiled. From shrews (Soricidae). Genotype *Amorphacarus elongatus* (Poppe, 1896) (syn. *Myobia elongatus* Poppe, 1896); other species, *A. hengererorum* Jameson, 1948.

Blarinobia, new genus

Leg I (Fig. 2, J) of four segments, with no claws; sometimes somewhat asymmetrical in the male. Legs II–IV each with a single claw. Some dorsal setae slightly expanded and weakly striated but not barbed. Body shape moderately broad. Penis with two loops. From the soricid shrew, *Blarina brevicauda*. Genotype is *Blarinobia simplex* (Ewing, 1938) (syn. *Myobia simplex* Ewing, 1938). Monotypic.

Afromyobia Radford, 1954

This genus was erected to hold *Myobia trouessarti* Poppe, 1896. It is distinguished by the long, flattened, striated setae on the venter; generally in the MYOBIIDAE such setae occur only on the dorsum. The fine illustrations of this species by Fahrenholz (1909) show that it is definitely close to *Eadica*. However, I have not examined specimens of *trouessarti*, and it is best to accept *Afromyobia*. Characters as in *Eadica*, except for the ventral setae as noted above. Genotype, *Afromyobia trouessarti* (Poppe, 1896) (syn. *Myobia trouessarti* Poppe, 1896). From the shrew, *Potamogale velox* Du Chaillu.

Radfordia Ewing, 1938

Leg I (Fig. 2, K) of three discernible segments, apparently with no terminal claws. Legs III and IV each with a single claw. At least some body setae expanded and weakly striated. Some setae with barbs. Body moderate for the family (neither broad as in *Protomyobia* and *Pteracarus* nor elongate as in *Neomyobia*). Penis slender and straight. Genotype is *Radfordia ensifera* (Poppe, 1896) (syn. *Myobia ensifera* Poppe, 1896). Also included are *R. subuliger* Ewing, 1938, *R. lemnina* (Koch, 1841), *R. ewingi* (Fox, 1937), *R. affinis* (Poppe, 1896), *R. multivaga* (Poppe, 1908), *R. lancearia* (Poppe, 1908), *R. zibethicalis* (Radford, 1936), *R. forcipiter* Lawrence, 1954, *R. aegyptica* Radford, 1951, *R. sigmodontis* Radford, 1951, and *R. rotundata* (Lawrence, 1951).

The genus *Cryptomyobia* has been proposed by Radford (1954) for *Myobia rotundata* Lawrence, 1951. The types, generously loaned to me by Dr. Lawrence, reveal that the male is a species of *Radfordia*, and that the female is actually a deutonymph, apparently of *Radfordia*. Dr. Lawrence (in a personal communication) stated that a true female, subsequently collected, is entirely different from the nymph and has a claw formula 2:1:1. Accordingly, *Cryptomyobia* must be synonymized with *Radfordia*.

The same circumstances obtain with the recently named genus *Austromyobia* Lawrence, 1954. This genus is based on *A. schoutedeni* described in the same paper; the "female" of *schoutedeni* are nymphs. The first and fourth legs illustrated resemble very closely those of nymphs of *Radfordia*, and *Austromyobia* is considered to be a synonym of *Radfordia*.

Myobia Heyden, 1826

Leg I (Fig. 2, D) as in *Radfordia*. This genus differs from *Radfordia* only in the nature of the second tarsus; in *Myobia* there is but a single claw on leg II. Actually the two genera are very close. All species from rodents. The genotype is *M. musculi* Schrank, 1781) (syn. *Pediculus musculi* Schrank, 1781). Also included are *M. oudemansi* Poppe, 1908, *M. otomyia* Lawrence, 1951, and *M. stewardi* Radford, 1948.

DISCUSSION

In general, the assignment of a species to a genus of MYOBIIDAE is not difficult; the species are fairly typical of one genus or another. However, the generic relationships are not always clear. There is no clearly traceable path from the more primitive genera to those believed to be more advanced, although some genera are clearly allied to each other.

In the MYOBIIDAE, the legs are believed to be good indicators of phylogenetic position, especially the segments of leg I and the claws on legs II, III, and IV. In

the more advanced genera, the terminal segments of leg I have become coalesced so that it is impossible to discern the original five segments typical of this group. With the fusion of segments in leg I there is a reduction or disappearance of the terminal claws of leg I. The first and second segments of leg I, although variable, are always distinct, and are easily recognized by their respective dorsal setae. The first segment bears a lateral seta, usually small, and an inner (or mesal) seta which is short, stout, and striated in some genera. Segment two of leg I always has two dorsal setae, the lateral being rather long; among the different genera there is little variation, and these setae are characteristic of this segment. Ventro-laterad the second segment is modified for clasping hair, this feature being rather characteristic of each genus. The third segment is always curved ventrad, forming a lateral hook; this hook occurs in all myobiid mites, and the third segment can invariably be recognized by this structure. The fourth and fifth segments may be distinct in the presumably primitive genera, or fused to a third segment in the advanced groups.

The claws of legs II, III, and IV may be similar and paired, markedly unlike, or single; the last condition is believed to be advanced. There is no great difference in these claws between sexes of one species; instances of sexual dimorphism with regard to these claws has arisen from a confusion of nymphs with females.

Other features that assist in judging the generic relationships are the penis and the general character of the body setae. These are discussed in the diagnosis for each genus.

The most primitive and least specialized genus is *Archemyobia;* leg I is rather long, the segments are distinct, and the terminal claws are quite large (Fig. 1, D). The two lateral spurs or projections are found elsewhere in this family only in nymphs of *Neomyobia* (Fig. 1, A) and possibly also *Calcarmyobia* and *Acanthophthirius.* The dorsal setae of *Archemyobia* (Fig. 1, C) are broad and bear conspicuous striations. The claws on leg II are subequal, but those on legs III and IV are quite unequal (Fig. 1, C). *Acanthophthirius, Calcarmyobia,* and *Neomyobia* together with *Archemyobia* form a group. They all possess similar dorsal setae, and *Neomyobia,* through leg I of its nymph, and in the claws of all legs, seems closest to *Archemyobia. Calcarmyobia* and *Acanthophthirius* are similar; in both genera, the inner seta of the first segment of leg I is short and striated, the leg segment itself is short and broad, and there is a reduction of tarsal claws on legs II, III, and IV. *Archemyobia* is found on a marsupial and it is not surprising that the most closely allied genera are parasites on bats (CHIROPTERA), another primitive group of mammals. One might expect that insectivores, also primitive mammals, would be hosts to mites related to *Archemyobia,* but this is so far not the case.

The next group to be considered occurs on insectivores; although they are similar in some ways to the above group of genera, the relationship is not close. This second group includes *Afromyobia, Eadiea, Eutalpacarus, Amorphacarus,* and *Blarinobia.* These genera are not characterized by any single group of characters, but nevertheless form a series in which the apparently most closely allied genera have features in common. From the descriptions of *Afromyobia trouessarti,* this species is similiar to *Eadiea,* but the details of leg I are not apparent, and can only be imagined. *Eadiea* possesses five segments in leg I; terminal claws are present. In *Eutalpacarus* only four segments are to be found in leg I; a pair of terminal claws occurs on the terminal part of this leg. These three genera (*Afromyobia,*

Eadiea, and *Eutalpacarus*) all possess a pair of claws on legs II, III, and IV; the first two genera are also similar in that at least some dorsal setae are expanded and conspicuously striated. In the general facies of leg I, *Eutalpacarus*, *Amorphacarus*, and *Blarina* resemble each other, but the terminal claws have been lost in the last two genera. In all three genera, the dorsal setae are slender or only slightly expanded and weakly striated. In *Amorphacarus* legs III and IV bear only a single claw, and in *Blarinobia* the claws on legs II are also reduced to a single claw. *Amorphacarus* and *Blarinobia* indicate an affinity in the asymmetrical development of leg I; this is striking in *Amorphacarus* and not uncommon in *Blarinobia*, especially in males. *Blarinobia* is the most specialized of this group of genera, and occurs only on the shrew, *Blarina brevicauda*.

The third group of genera consists of *Protomyobia* and *Pteracarus*. In each, leg I is of five segments, and the last segment bears a pair of terminal claws; in *Protomyobia* this leg is somewhat longer, but the similarity is obvious (Fig. 2, E and F). In both genera the body shape is broad and short, and the setae are all slender and non-striated. In *Protomyobia* there is a pair of claws on leg II, but a single claw on leg III and IV. In *Pteracarus* legs II, III, and IV each bear a pair of stout curved claws. Leg I of these two genera is rather unspecialized for the family, but *Protomyobia* and *Pteracarus* seem not closely allied to any other genera. *Protomyobia* occurs only on shrews and *Pteracarus* only on bats.

The last group of genera (*Radfordia* and *Myobia*) is by far the most specialized and is not at all closely related to any genera in the first three groups. *Radfordia* and *Myobia* are virtually the same in the nature of leg I (Fig. 2, K). The third, fourth, and fifth segments are completely fused, no separation being apparent; there are no terminal claws on leg I. In *Radfordia* there is a pair of terminal claws on leg II, and single claws on legs III and IV. In *Myobia* legs II, III, and IV each have a single claw. *Myobia* and *Radfordia* occur only on rodents.

The most primitive and least specialized of MYOBIIDAE, *Archemyobia*, parasitize primitive, non-placental mammals, the MARSUPIALIA. Two groups of genera occur on primitive placental mammals, the CHIROPTERA and INSECTIVORA. The most specialized and modified of myobiid mites, *Radfordia* and *Myobia*, occur on RODENTIA, the most advanced mammals from which myobiid mites are known.

Key to Genera of Myobiidae

(Adults)

1. Leg II with a single tarsal claw 2
   Leg II with paired tarsal claws 3
2. Leg I of 3 segments; segment III not broader than segment II with no terminal claw; Laterals I expanded and striated; penis straight; capitulum and Legs I symmetrical ..... *Myobia*
   Leg I of 4 segments, segment III much broader than segment II (apparently 5-segmented in males) with a terminal claw; Laterals I not expanded and with faint striae, if any; penis in a double coil; capitulum and Legs I sometimes asymmetrical ..... *Blarinobia*
3. Legs I and capitulum asymmetrical (esp. males) ..... *Amorphacarus*
   Legs I and capitulum symmetrical 4
4. Legs III and IV with a single terminal claw 5
   Legs III and IV with paired terminal claws 7
5. Dorsal setae rather slender, not striated; Leg I with 5 distinct segments and 2 terminal claws; penis rather stout, irregular, with a basal hook ..... *Protomyobia*

At least some dorsal setae expanded and striated; Leg I of fewer than 5 distinct segments and with one or no terminal claw; penis straight 6

6. Leg I of 4 segments; segment II being narower than I or III; body long; dorsal setae expanded and heavily striated, but not barbed ..... *Acanthophthirius*
Leg I of 3 segments (segments III, IV, and V being united), segment II being broader than I or III; dorsal setae various, but some with barbs ..... *Radfordia*

7. First segment of Leg I with a dorsal, striated, peglike projection ..... *Calcarmyobia*
First segment of Leg I with no peglike projection 8

8. With dorsal setae slender and barbed, but neither expanded nor striated; claws on Legs III and IV stout and markedly recurved; Leg I of 5 distinct segments *Pteracarus*
With at least some dorsal setae expanded and/or striated, but none barbed; claws on Legs III and IV various but not stout and markedly recurved 9

9. Claws on Legs III and IV markedly unequal, one 5 times the length of the other; Legs I with several conspicuous lateral and mesal projections, and with a pair on large terminal claws ..... *Archemyobia*
Claws on Legs III and IV of more or less equal length; Legs I with no lateral projections; claws on Leg I, if present, rather inconspicuous 10

10. At least some dorsal setae with small but conspicuous bulbous expansions, not striated; Laterals I borne on a pair of sclerotized plates ..... *Eutalpacarus*
Setae not expanded as above, and Laterals I not borne on plates 11

11. Almost all dorsal setae expanded and striated; body long (ca 5 times as long as wide); penis sinuate, but not coiled or doubled ..... *Neomyobia*
Laterals I and sometimes some other dorsal setae expanded and striated; body short and broad (ca 2 times as long as wide) 12

12. Some ventral setae expanded and striated ..... *Afromyobia*
No ventral setae expanded and striated ..... *Eadica*

It is a pleasure to thank Dr. R. F. Lawrence and Dr. C. S. Radford for the loan of specimens from their collections. Dr. G. Owen Evans has kindly sent me some notes regarding the type of *Acanthophthirius etheldredae* in the British Museum. Financial assistance in this study was received from the Dryden Biological Laboratory.

SUMMARY

Archemyobia inexpectatus, new genus, new species, is described from the opossum, *Didelphis virginiana*, and is the first record of a myobiid mite from a marsupial. An evaluation of the proposed generic names for myobiid mites indicates the need for some adjustments: *Foliomyobia* Radford and *Binuncus* Radford are synonyms of *Neomyobia* Radford; *Ewingana* Radford is a synonym of *Acanthophthirius* Perkins; *Cryptomyobia* Radford and *Austromyobia* Lawrence are synonyms of *Radfordia* Ewing; and *Blarinobia* is proposed as a new genus for *Myobia simplex* Ewing.

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PEREZIA FUMIFERANAE N. SP., A NEW SPECIES OF MICROSPORIDIA FROM THE SPRUCE BUDWORM *CHORISTONEURA FUMIFERANA* (CLEM.)*

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In 1947, the Forest Insect Laboratory, Sault Ste. Marie, Ontario, began investigations of the disease organisms present in certain forest insects, including the spruce budworm, *Choristoneura fumiferana* (Clem.). In a survey of the pathogens present in the spruce budworm in Ontario, Bird and Whalen (1949) reported the presence of viruses, fungi, bacteria, and a protozoan of the order MICROSPORIDIA. Subsequent surveys indicated that the last organism was present in varying degree in most budworm populations throughout the Province. An investigation into the biology of this parasite was undertaken in 1952 with the aim of its possible use as a biological control factor against the spruce budworm. The first problem was to determine the identity and life cycle of the microsporidian, and it is these topics which are considered in this paper.

MATERIAL AND METHOD

The spruce budworm, a native North American insect, overwinters as a second instar larva in a silken hibernaculum. It emerges in early May to commence feeding upon the new growth of spruce and balsam fir. Some of the insects used in this study were reared under laboratory conditions but much of the host material was obtained directly from field populations in the vicinity of Black Sturgeon Lake Field Station, north of Port Arthur, Ontario. The laboratory reared insects were induced to emerge from the hibernacula and were fed balsam buds that had been picked the previous spring and preserved by freezing.

The original stock of microsporidian spores was obtained from the Port Arthur and Algonquin districts of Ontario. Since the MICROSPORIDIA are obligate parasites, fresh spores can be obtained only by periodically infecting larvae and allowing the disease to develop to completion.

The insects were infected experimentally by permitting them to feed on balsam buds contaminated with a water suspension of spores. Stages in the life cycle of the microsporidian were studied by killing the insects at intervals and examining fresh preparations by light, dark-field, and phase microscopy, smears stained with Giemsa, and sections stained in Mayer's haemalum.

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The study of the life stages of the MICROSPORIDIA presents some difficulties as these organisms are obligate intracellular parasites. Direct observation of living parasites within the host cells appears to be impractical; the worker must resort to stained sections and smears of infected tissue. In addition, shortly after infection, almost all the developmental stages may be found simultaneously, making it very difficult to ascertain their proper sequence. Hence the life cycle suggested for this species is derived by arranging the observed stages in a logical order based on present knowledge of cell division and nuclear changes and aided whenever possible by the observed chronological sequence of the various stages.

The polar filament is a characteristic structure of the microsporidian spore. The length of the filament and the factors which cause its extrusion vary considerably among species. In the species under study extrusions result from pressure on the cover glass but the polar filaments thus obtained are coiled tightly in the vicinity of the spore rendering measurement of their lengths impossible. Of the many chemical reagents tested only hydrogen peroxide was found to be effective and this only when the spores were in a clean suspension.

PATHOLOGY

Among laboratory-reared insects, the principal site of infection is the cells of the midgut. The Malpighian tubules are frequently involved and occasionally small areas of muscle tissue are invaded. However, in naturally-infected insects many more tissues are infected: the fat body, silk glands, epidermis, gonads, hind gut, and nerve tissue. It is suggested that these differences are the result of the mode of infection. Laboratory-infected insects receive large doses of spores and die within a short time, whereas naturally-infected insects receive only small numbers of spores and hence live considerably longer, thus allowing the parasite time to spread throughout the body. Because the midgut cells are the principal site of infection in both cases, it is postulated that it is here that the parasite exerts a lethal effect.

As in all species of MICROSPORIDIA, only the cytoplasm of the host cells is invaded by the parasite, but, unlike most other species, neither the cytoplasm nor the nucleus of the infected cell becomes hypertrophied. The majority of the infected cells retain their shape until after death occurs, but some apparently rupture as spores may be found in both frass and regurgitated material during the later stages of infection.

The disease does not produce any apparent external symptoms; even for several hours after death infected larvae appear normal.

Young larvae at room temperatures die within 7 or 8 days after infection, but fifth- and sixth-instar larvae are sometimes able to survive extremely heavy infections.

Perezia fumiferanae n. sp.

SCHIZOGONY

By starting at the spore stage, which is easily identified and known as the end-point of the cycle, and retracing the cycle step by step, the following sequence of stages appears probable.

The period of schizogony appears to be one of constant growth and multiplication. All forms have two or more nuclei. No indication of motility has been observed among these organisms in the living state.

The first forms observed in infected larvae are very small schizonts (Plate I, fig. a) measuring about 1 micron in diameter. They contain two compact nuclei that stain very deeply by the Giemsa method; only a small amount of lightly stained cytoplasm is visible. The multiplication of these and all succeeding schizont forms occurs by several methods. The most common method is simple binary fission, the nuclei divide first into a tetranucleate form (Plate I, figs. b, f, k) which in turn cleaves into two binucleate forms (Plate I, figs. c, g, i). Occasionally the nuclear division is independent of cleavage resulting in the formation of chains of cells (Plate I, fig. d). These chains measure 8–10 microns in length but rarely comprise more than four cells. Another form of multiplication involves the production of schizonts containing as many as 12 nuclei (Plate I, fig. e). The nuclei are arranged loosely in pairs scattered throughout the irregularly staining protoplasm. New binucleate schizonts are produced by budding (Plate I, fig. m) or fragmentation (Plate I, fig. n) of the multinucleate form. This type of multiplication may occur at any stage during the schizogonic period.

As the schizonts gradually increase in size, their nuclei stain less deeply and frequently develop clear centers. The ultimate stage appears to be a large, rounded, binucleate form with a vacuolated cytoplasm (Plate I, fig. o). This form measures 5 microns in diameter and is apparently the stage that becomes transformed into the early sporont.

SPOROGONY

The sporogonic stages are all larger and more elongate than the schizogonic stages. The nuclei stain well and usually have a clear area in the center. The cytoplasm is generally streaked and mottled, and stains very lightly.

The early sporont is a binucleate crescent-shaped body about 7 microns in length (Plate I, fig. p). The nuclei divide to form a tetranucleate sporont which is pyriform in shape and usually has the smaller end slightly curved (Plate I, fig. r). Two nuclei migrate to each end of the elongating cell (Plate I, fig. s) and a constriction develops at the midline (Plate I, fig. t). As the constriction becomes more pronounced the sporont assumes the characteristic bilobed appearance shown in Plate I, fig. u. Two sporoblasts result from the division of the sporont. The sporoblasts are about 5 microns long, each containing two nuclei which invariably lie parallel to the short axis of the cell (Plate I, fig. v). The cytoplasm of the sporoblast becomes more compact, the nuclei tend to become rounded, and a spore wall appears (Plate I, fig. w). As it matures the spore contracts further and the nuclei fuse. The mature spore contains only a single indistinct rod or U-shaped nucleus and faintly-stained cytoplasm (Plate I, fig. x).

When fixed and stained the mature spore measures 2 by 3–4 microns and when in the living state 2 by 3–5 microns. The spores are variable in shape: some are ovoid, others have one end slightly larger than the other, and many are slightly curved, but the majority have straight parallel sides with symmetrically rounded ends. Fresh spores are highly refractile and little of the internal structure is discernible apart from a large vacuole at one end (Plate I, fig. y). The size of this vacuole is quite variable and may occupy from 25 to 75 percent of the volume of the spore. Double spores, presumably formed from incompletely separated sporoblasts, are extremely rare. Although many workers have reported the presence of microspores and macrospores in various MICROSPORIDIA, in this species there is a com-

TABLE I.—*A comparison of the more important characteristics of the described species of the genus* Perezia

	P. lankesteriae Leger & Duboscq 1909	*P. trichopterae* Weiser 1946	*P. legeri* Paillot 1918b	*P. mesnili* Paillot 1918a	*P. pieris* Paillot 1924	*P. pyraustae* Paillot 1928	*P. fumiferanae*
Host: Order Species	GREGARINA *Lankesteria ascidae*	TRICHOPTERA *Plectrocnemia geniculata*	LEPIDOPTERA *Pieris brassicae*	LEPIDOPTERA *Pieris brassicae*	LEPIDOPTERA *Pieris brassicae*	LEPIDOPTERA *Pyrausta nubilalis*	LEPIDOPTERA *Choristoneura fumiferana*
Tissue parasitized	Entire host	—	fat body, giant cells	silk glands, Malpighian tubes	Malpighian tubes, silk glands, digestive tract	Malpighian tubes, silk glands	most tissues, esp. midgut, fat body
Spore shape	ovoidal	ovoidal	ovoidal	ovoidal	elongate oval	ovoidal	subcylindrical
Spore size: (in microns) length	2.5	3.5–4.0	4.0–5.0	3.4	5.0–6.0	3.5–6.0	3.0–5.0
width	—	2.0	—	1.5–2.0	—	1.8–3.0	2
Polar filament length (microns)	—	200–340	30–40	18–20	—	30–65	60–105
Maximum no. of nuclei*	12	—	8	8	4	4	12

* Present in polynuclear schizonts.
— Information unknown or unavailable.

plete gradation from the smallest to the largest spore. The length of the polar filament, extruded under the stimulus of hydrogen peroxide, varies from 65 to 105 microns, the average length being 80 microns.

Photographs of certain stages described above are shown in Plate II.

DISCUSSION

As each sporont of this microsporidian forms only two spores and as the infected host cells do not become hypertrophied, it qualifies for inclusion in the genus *Perezia* Leger and Duboscq. A survey of the literature reveals that only six species of *Perezia* have been reported. Of these, five are from the INSECTA and one from the PROTOZOA. It should be mentioned that several of the species are incompletely described and hence are difficult to compare with the present species. Some of the more important characteristics of these species are compared with those of the budworm parasite in Table I.

Early in the course of these investigations, it was noted that the budworm microsporidian was similar in some reports to *P. pyraustae* Paillot, which was redescribed by Hall (1952). Fresh spores of *P. pyraustae* were obtained from the U.S.D.A. European Corn Borer Research Laboratory at Des Moines, Iowa. The spores were fed to 15 spruce budworm larvae, while an equal number of larvae were fed spores of the species described in this paper. At the end of 10 days all insects fed spores of the budworm microsporidian were heavily infected, whereas those fed *P. pyraustae* showed no signs of infection.

A comparison of the characteristics of the present organism with those of all the described species of *Perezia* shows no significant agreement. It is apparent that the budworm microsporidian is a new species and the name *Perezia fumiferanae* is herewith proposed.

SUMMARY

A new species of MICROSPORIDIA, *Perezia fumiferanae* has been found parasitizing the spruce budworm *Choristoneura fumiferana* (Clem.). The life cycle of the protozoan is described and some details are given of its effect on the host.

ACKNOWLEDGMENTS

I would like to thank Dr. R. R. Kudo, Institute of Microbiology, Rutgers University, for reading and criticizing the manuscript. Thanks are also due to the staffs of the Forest Insect Laboratory and the Laboratory of Insect Pathology, Sault Ste. Marie, for their assistance in obtaining infected host material.

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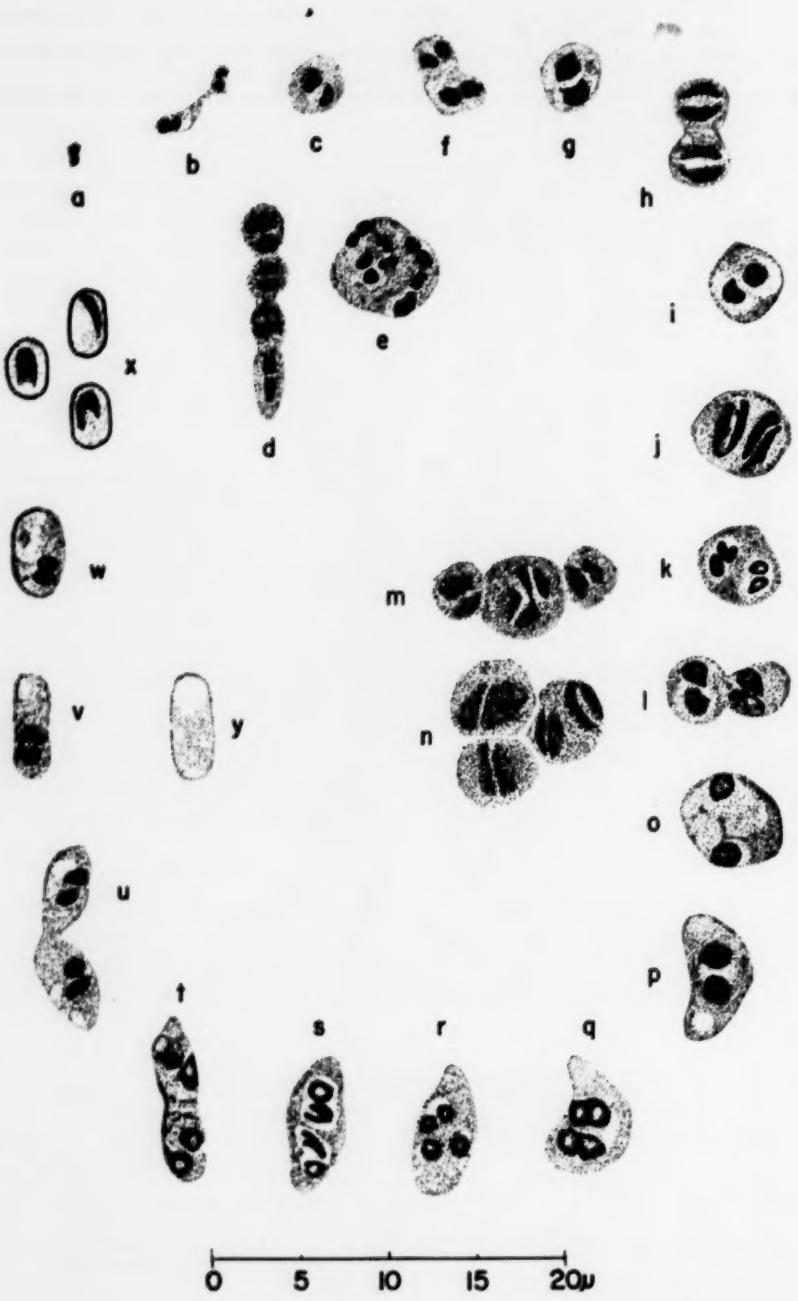
EXPLANATION OF PLATES

PLATE I

Stages in the life cycle of *Perezia fumiferanae* n. sp.
FIGS. a–o. Schizonts.
FIGS. p–u. Sporonts.
FIG. v. Sporoblasts.
FIG. w. Young spore.
FIG. x. Mature spores.
FIG. y. Fresh spore.

PLATE I



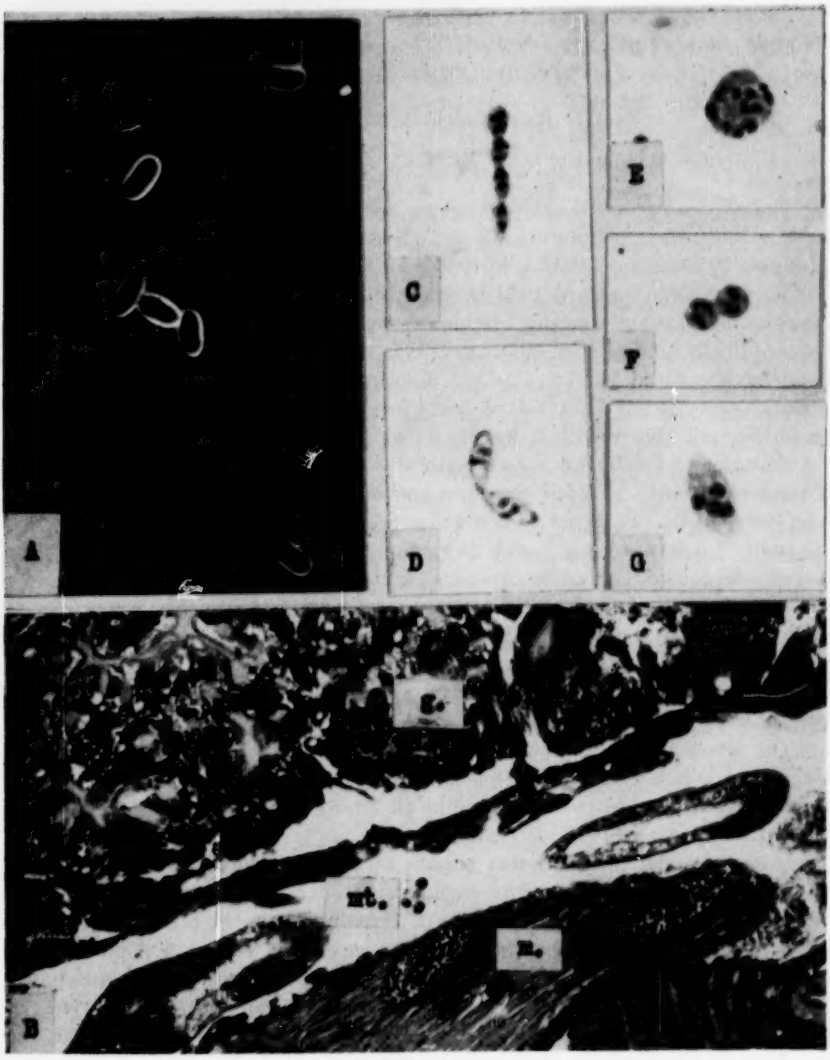


PLATE II

Photographs of *Perezia fumiferanae* n. sp.

FIG. A. Unstained spores in water suspension (×1850).

FIG. B. Section of infected budworm larva. Spores in midgut epithelium (g), muscle (m), and Malpighian tubule (mt.); haemalum stain (×470).

FIG. C. Chain-form schizont; Giemsa stain, (×1850).

FIG. D. Sporont dividing to form two sporoblasts; Giemsa stain, (×1850).

FIG. E. Multinucleate schizont; Giemsa stain, (×1850).

FIG. F. Dividing schizont; Giemsa stain, (×1850).

FIG. G. Early sporont; Giemsa stain, (×1850).

STUDIES ON THE LIFE-HISTORY AND BIOLOGY OF *OOCHORISTICA DESERTI* N. SP. (CESTODA: LINSTOWIIDAE) FROM DESERT RODENTS[1, 2]

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The life-cycles of the cyclophyllidean cestodes of the super-family ANOPLOCEPHALOIDEA were unknown until 1937 when life-histories were elucidated for *Moniezia* by Stunkard (1937 a, b, 1938), for *Cittotaenia*, (Stunkard, 1939, 1941), and for *Bertiella*, (Stunkard, 1940). Since the studies of Stunkard, life-cycles in the ANOPLOCEPHALINAE involving exclusively oribatid mites have been completed successfully by numerous investigators. This work has been summarized in tabular form by Kates and Runkel (1948). Subsequently, Freeman (1952a) reported oribatid mites as the intermediate hosts for *Monoecocestus americanus*, and *M. variabilis;* and Melvin (1952) for *M. sigmodontis.*

Potemkina (1944b) has shown oribatid mites to be the intermediate hosts for *Thysaniezia giardi.* This is the only known life-history for the thysanosomine anoplocephaloids. It should be noted that Spasskii (1951) placed *T. giardi* in the subfamily THYSANIEZIINAE under the family AVITELLINIDAE, but it is not possible to evaluate this revision of the ANOPLOCEPHALOIDEA at this time.

The life-cycle of *Oochoristica symmetrica* [*syn. Mathevotaenia symmetrica* of Akhumian (1946)] was demonstrated successfully by Rendtorff (1948) who obtained development of the cysticercoids in the larvae and adults of coleopteran and lepidopteran insects, but not in oribatid mites. The life-history reported in the present work brings to three the number of known life-cycles in the LINSTOWIIDAE.

The life-cycle reported by Rendtorff for *Oochoristica symmetrica* involves the utilization of insects as the intermediate host for this cestode, whereas, oribatid mites serve as the intermediate host in all known life-histories of the ANOPLOCEPHALIDAE.

It was thought, therefore, that a study of the life-history of a new species of *Oochoristica* from desert rodents would be of interest and would perhaps substantiate further the role of insects as intermediate hosts in linstowiid cestodes.

Freeman (1952b) studied the effect of temperature on the development and size of *Monoecocestus* larvae in oribatid mites. His is one of the few precise studies on the effect of temperature on anoplocephalid larval development. During the course of the present study the writer noted a distinct effect of temperature on the developmental rate of cestode larvae. It seemed desirable, therefore, to study this phenomenon under controlled conditions.

MATERIALS AND METHODS

Intermediate Hosts

Six genera of insects and one genus of mites were tested as intermediate hosts

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for the cestode. The insects represented two orders, LEPIDOPTERA and COLEOPTERA. Of the COLEOPTERA studied, three species, *Tribolium confusum, Tenebrio molitor,* and *Gnathocerus cornutus* belong to the TENEBRIONIDAE, and two species *Attagenus piceus* and *Dermestes maculatus* to the DERMESTIDAE. One lepidopteran species, *Ephestia cautella,* was studied.

The acarine studied was a free-living mite of the family ACARIDAE (syn. TYROGLYPHIDAE), but was not identified to genus.

The insects were obtained from established laboratory cultures or from contaminated grain stores. Each species was maintained in isolated, covered culture dishes free from contamination by rodents.

Mites obtained from contaminated food stores were kept in shell vials containing dried prunes kept moist by the addition of a few drops of 10% sucrose solution.

The ova used in the feeding experiments were obtained from worms removed from freshly killed animals. Approximately the last two gravid proglottids were removed from the large intestine of the host, placed on a piece of filter paper, teased apart, and moistened with water. The segments were aged from 1 to 5 days at room temperature prior to their use. Larval and adult stages of the arthropods, previously starved for 24 to 48 hours, were placed in petri dishes with the infective material. The proglottids were completely eaten by the larger insects within 12 hours; the smaller insects and mites required a somewhat longer time, usually 24 hours. At this point, the arthropods were transferred to petri dishes containing cereal or animal matter to await future examination.

Examination of the insects and mites for the larval stages of the cestode was carried out as follows: the insects were placed on a slide in a few drops of physiological saline, dissected, a cover slip added, and the preparation examined under the microscope. The mites are transparent, and with slight cover glass pressure, could be sufficiently flattened, without rupturing the internal organs, to allow adequate examination. Measurements of the cestode larvae were taken as soon as possible after the dissection.

After the life-cycle had been completed successfully using *Tribolium* as an intermediate host, this insect was used as a control in all subsequent experiments involving other arthropods.

Definitive Hosts

Two species of kangaroo rats, *Dipodomys m. merriami* and *D. panamintinus mohavensis,* one ground squirrel, *Citellus leucurus,* one pocket mouse, *Perognathus longimembris,* hamsters, white and hooded rats, and white mice were used as definitive hosts for the completion of the life-cycle. In all cases the wild rodents used in these experiments were trapped in the field and maintained in the laboratory. Attempts to rear laboratory-born young to maturity in the case of the wild rodents were unsuccessful. The following procedure was adopted to determine, with a minimum chance of error, whether the wild animals to be used in infection experiments were already harboring cestodes when brought into the laboratory.

In every case, the feces of each animal was checked periodically by direct smear for tapeworm ova at least one month prior to the experimental feeding of the cestode larvae. In most cases animals were kept 1 to 4 months and in four cases 9 to 11 months in the laboratory prior to feeding of the larvae. The direct fecal

smear was considered a valid technique for the detection of adult cestodes. Almost invariably two smears were sufficient to detect the presence of adult cestodes, even when subsequent autopsy showed only one worm to be present. All necessary precautions then were taken to prevent unintentional laboratory infections of animals shown to be negative for adult tapeworms by the above method.

Kangaroo rats acted as controls in all infection experiments involving the other animals.

The laboratory-reared albino rats and mice, hooded rats, and hamsters to be used in the infection experiments were isolated and maintained free from insect contamination.

Preparation of Cestode Material

Adult and larval specimens of the cestode were relaxed by placing them in tap water in the refrigerator for 12 to 24 hours. After fixation in Bouins' fluid, the specimens were stained with a dilute solution of Mayer's acid haemalum (Lillie modification). A dilution of five to ten drops of stock solution to 100 cc. of distilled water with a staining time of 24 to 48 hours gave the best result. De-staining was unnecessary with such a dilute solution. Specimens were cleared in toluene and mounted in permount.

Sections of the intestine from the infected insects, and of mature proglottids were made using the standard paraffin techniques, and stained with Harris' haematoxylin and eosin.

This new species was compared with other species of *Oochoristica* from mammals by consulting original descriptions and those in the reviews of Baer (1927) and Hughes (1940). The comprehensive work of Spasskii (1951), which is published in Russian, is cited, but was not available to the writer for study.

The following description is based on specimens recovered from naturally infected *Dipodomys m. merriami,* and *Citellus leucurus,* and experimentally infected *Dipodomys m. merriami, D. panamintinus mohavensis, Citellus leucurus,* and *Perognathus longimembris.* (All measurements in millimeters).

Oochoristica deserti, n. sp., adult
(Plate II, figs. 10–14)

Diagnosis: Strobila 55–345 long, 0.68–2.39 wide at level of semigravid proglottids. Proglottids 87–363 in number. Scolex unarmed, 0.26–0.50 wide (Fig. 11). Suckers 0.066–0.126 in diameter. Neck approximately 0.8–2.7 long. Genital pores alternating irregularly, situated in anterior third of proglottid (Fig. 14). Mature proglottids 0.50–1.09 long by 0.59–1.46 wide. Excretory system consisting of two pairs of longitudinal ducts, presence or absence of cross connections, or ramifications of these ducts, not determined. Genital ducts passing dorsal to both longitudinal excretory ducts.

Sub-cuticular musculature poorly developed. Parenchymal musculature consisting of two layers; inner layer separated by about 0.034 from outer layer (Fig. 10). Inner layer consisting of isolated longitudinal bundles of small numbers of fibers, bundles separated by as much as 0.093 in mature proglottids. Outer layer more weakly developed, consisting of isolated longitudinal bundles of few or single fibers.

Testes 18–60, 0.031–0.088 in diameter; in two layers, lateral and posterior to female reproductive organs, usually continuous behind yolk gland, numbers in poral and aporal halves of proglottid approximately equal (Fig. 14). Cirrus-sac 0.088–0.187 long by 0.046–0.099 wide, extending to inner longitudinal excretory canal. Vas deferens moderately tortuous, running inward to an anteromedial position, then turning posteriorly, not traceable farther (Fig. 14).

Ovary 0.188–0.600 wide in mature proglottid; bilobed, each lobe consisting of many finger-like projections, aporal lobe slightly more anterior than poral lobe (Fig. 14). Yolk gland, multilobed, posterior to central part of ovary, 0.083–0.330 wide. Vagina opening posterior to cirrus-sac,

passing inward from genital pore as nearly straight tube to anteromedian position then turning posteriorly, eventually terminating at Mehlis' gland. Seminal receptacle not observed. Uterus ephemeral as discrete structure, beginning as faintly observable network anterior and lateral to reproductive organs in last few mature proglottids (Fig. 14), almost immediately breaking down into uterine capsules 0.142–0.218 by 0.102–0.165 in diameter, each capsule enclosing one egg. Egg with thin outer shell, 0.039–0.058 by 0.034–0.046 (Fig. 12); onchosphere including embryonic shell, 0.031–0.051 by 0.027–0.034; onchosphere hooks 0.014–0.017 long (Fig. 13). (Eggs and onchospheres measured from living material.)

Hosts: Dipodomys merriami merriami Mearns (type); *Dipodomys panamintinus mohavensis* (Grinnell); *Citellus leucurus* (Merriam); *Perognathus longimembris* (Coues).

Location: Small intestine.

Locality: Victorville, San Bernandino County, California.

Holotype: U. S. Nat. Mus. Helm. Coll. No. 37422.

Paratypes: In collection of author.

There are over 21 valid species of *Oochoristica* parasitic as adults in mammals. Spasskii (1951) listed 41 species of *Oochoristica,* and placed 19 species including *erinacei* and *symmetrica* in the genus *Mathevotaenia* Akhumian (1946), of which the latter species was considered the type. No decision is made here as to the status of the genus *Mathevotaenia.* However, if later study of the work of these Russian authors validates Akhumian's new genus, then *O. deserti* should be transferred thereto. Joyeux (1927) described *O. erinacei* var. *rodentium* from the gerbil, *Meriones shawi,* in Algeria. This form differs from *O. erinacei* only in the length of the cirrus-sac, total length, and host. *O. erinacei* is a parasite chiefly of carnivores, whereas the worm described by Joyeux is from a rodent. This difference, as noted by Joyeux, may be merely a case of accidental parasitism by *O. erinacei.* Length is quite variable in the genus *Oochoristica,* so that the principal difference between the two worms lies in length of cirrus-sac. This difference does not warrant the erection of a new variety, and *O. erinacei* var. *rodentium* should be regarded as a synonym of *O. erinacei.*

The species of *Oochoristica* from California dessert rodents differs from all other species of *Oochoristica* parasitic in mammals, other than rodents, in the following principal ways: size of cirrus-sac; size of scolex; number of testes; length of cirrus-sac; size of suckers; position of the vaginal opening with respect to the cirrus-sac; and arrangement of the gonopores.

The new species resembles most closely the three species of *Oochoristica* previously known from rodents, and in particular *O. symmetrica.* It differs from *O. dipi* (Parona, 1900) in having (1) smaller suckers, (2) fewer testes, (3) shorter cirrus-sac, and (4) chiefly in the arrangement of the testes which in *O. dipi,* are mainly anterior to the ovary and antiporal in distribution, whereas in the new species the testes are lateral and posterior to the ovary and of equal number in the poral and aporal halves of the proglottid.

From *O. erinacei* Meggit, 1920, (including *O. erinacei* var. *rodentium*) it differs in having (1) a wider cirrus-sac, (2) smaller suckers, (3) slightly larger eggs, and (4) mainly in the length of the cirrus-sac which in *O. erinacei* extends past the excretory ducts as far as half way across the segment, whereas in the new species, the cirrus-sac extends only as far as the inner longitudinal excretory duct.

From *O. symmetrica* (Baylis, 1927) it differs in having (1) slightly smaller suckers, (2) the testes arranged in two layers instead of mainly in one layer as in *O. symmetrica,* (3) a cirrus-sac which tends to be wider, (4) a slightly smaller ovary, (5) genital ducts passing dorsal to both longitudinal excretory ducts instead

of between as in *O. symmetrica,* (6) cirrus-sac extending to the inner longitudinal excretory duct, and (7) testes tending to be fewer in number.

In making the above comparison, the author has examined and taken measurements from the type specimen of *O. symmetrica* kindly loaned by Mr. Prudhoe of the British Museum. My measurements agree with those given by Baylis in the original description of *O. symmetrica.*

Although the new species resembles *O. symmetrica* in many ways, I consider that the differences existing between the two species are of taxonomic importance. The measurements of the new species were taken from approximately 35 specimens. The size ranges given for each structure in some cases represent approximately 300 measurements. In addition, the new species is based on specimens taken from 4 host species and from natural and experimental infections. It is believed that the number of measurements taken for each structure is adequate, and that they represent the morphological variability in this species in so far as such variability may be affected by type of host, and laboratory vs. natural conditions.

Results from life-cycle experiments, lend further support to the concepts of *O. symmetrica* and *O. deserti* as distinct species. Rendtorff (1948) was able to infect laboratory albino rats and mice with the larvae of *O. symmetrica.* In repeated trials I have been unable to infect these animals with the larvae of the new species.

The evidence presented is sufficient to warrant the recognition of a new species for the worms from California desert rodents.

LIFE-HISTORY STUDIES

Intermediate Hosts Infected

The results obtained by feeding the onchosperes of *Oochoristica deserti* experimentally to arthropods are presented in Table I. A negative result indicates

TABLE I.—*Results of feeding onchospheres of* Oochoristica deserti *to arthropods*

Species	Life stages infected*	Life stages examined*	Cysticercoids
Acarid mite	A	A	absent
Gnathocerus cornutus	A	A	present
Tribolium confusum	A	A	present
	L	A	present
Tenebrio molitor	A	A	present**
	L	L, A	absent
Attagenus piceus	A	A	absent
	L	L, A	absent
Dermestes maculatus	L	A	present
Ephestia cautella	L	A	present

* L—larva, A—adult.
** One positive beetle out of 60 dissected.

failure of the onchospheres to develop in that species of arthropod upon repeated trials in which *Tribolium confusum* adults served as the controls.

One adult *Tenebrio molitor* was found infected with a larval stage comparable to that shown in Fig. 5. Subsequent attempts to obtain development of the cysticercoid in this insect were unsuccessful. Rendtorff (1948) also was unable to infect *T. molitor, T. obscurus,* and the cockroach, *Blatta orientalis* with the onchospheres of *O. symmetrica.* It may be that the tissues of these insects are generally impenetrable to the embryos of these tapeworms, and only on rare occasions will the embryos succeed in developing in these insects. The ability of onchospheres to penetrate into the body cavity of the intermediate host is de-

pendent in part upon the cutting action of the hooks, and in part, perhaps, upon the secretions from the penetration glands. The intestinal wall in *Tenebrio* and *Blatta* is relatively thick as compared to that of the other insects used in this study and in that of Rendtorff. It may well be that secretions from the penetration glands are spent before the embryo is able to gain entrance to the body cavity of the insect.

The one case in which a larva did develop in *Tenebrio*, suggests that the hemocoel of this insect is physiologically suited for the growth and development of the cysticercoid, and its general refractoriness to infection may be attributed to the mechanical barrier imposed by the relatively thick intestinal wall.

The writer was unable to infect *Attagenus piceus* with the onchospheres of *O. deserti*, although Rendtorff infected this insect successfully with the onchospheres of *O. symmetrica*. Considerable difficulty was experienced in the handling of this insect in infection experiments. The adults were short-lived and frequently died before examination. The larvae were torpid and showed no desire, even when starved, to ingest the onchospheres. The lack of success in infecting this beetle may be due to their failure to feed on the proglottids, rather than the inability of the onchospheres to develop in the insect.

The number of cysticercoids harbored by the host apparently is dependent on the size of the insect. *Dermestes* adults frequently contained over 30 fully developed cysticercoids. *Tribolium*, a much smaller insect, usually averaged 6–15 cysticercoids.

Penetration Glands in the Onchosphere

Reid (1948) by the use of vital stains and phase microscopy demonstrated clearly for the first time, the presence of penetration glands in the onchospheres of cyclophyllidean cestodes. Penetration glands are known to be present in the larval stages of digenetic trematodes and the procercoid larvae of pseudophyllidean cestodes. It is not surprising, therefore, that such structures should be present in other cestodes whose embryos also must penetrate to varying degrees the tissues of the intermediate host.

The penetration glands in the onchospheres of *Raillietina cesticillus* are shown in Figure 16, taken from Reid (1948). The two glands, connected posteriorly by an isthmus, extend almost the entire length of the animal. A single nucleus is seen within each gland. The secretory pores of the glands are located slightly dorsolateral to the middle pair of hooks. Reid observed the glandular secretion exuding from the pores in the form of droplets.

The glands open near a point where the hooks come together before starting their backward stroke. Reid has postulated that the secretion from these glands is either histolytic in action, or perhaps by adhesive properties may aid the hooks in gaining a firmer attachment on the mucosa of the intermediate host. Another possibility is that the secretion may affect the inter-cellular cement through the action of hyaluronidase since this substance has been found in the secretions of schistosome cercariae (Kuntz, 1953).

I used both neutral red, in a concentration of one part of dye to 10,000 parts of distilled water, and the Spencer phase microscope (dark and light contrast, high dry objectives) in an effort to demonstrate penetration glands in the onchospheres of *O. deserti*. As a control, *Hymenolepis diminuta* embryos were studied in which the glands could be demonstrated vividly with neutral red.

Rendtorff (1948) noted what he termed "elliptical areas" in the embryos of *O. symmetrica* after four days within the insect. He was able also to demonstrate these structures in the onchospheres by means of vital staining. Similar areas were observed by me in the embryos of *O. deserti* after two days within the insect (Fig. 4).

Phase microscopy failed to reveal the presence of penetration glands in the onchospheres of *O. deserti.* The glands could be demonstrated, however, with neutral red staining (Fig. 15; cf. Fig. 16). The isthmus was demonstrable, but the anterior extent of the glands and the secretory pores could not be observed.

There is no doubt that the structures observed by Rendtorff and the writer in the onchospheres and embryos of *O. symmetrica* and *O. deserti,* respectively, are the penetration glands described by Reid. The glands do not appear to be as well differentiated as those observed in the onchospheres of *H. diminuta.*

As noted by Reid, the ability to demonstrate the glands is dependent on the size of the onchospheres. He was able to demonstrate readily the glands in *Raillietina* embryos which, like *Hymenolepis,* are relatively large. On the contrary, in the smaller embryos of *Moniezia,* demonstration of these glands was more difficult. The embryos of *Oochoristica* also are relatively small, which may account for the difficulty in demonstrating the glands in this cestode.

Development in the Intermediate Host

The following discussion is based on study of the development of the cysticercoids in adult *Tribolium confusum* maintained at 30° C. Essentially the same pattern of development was obtained in the other insects. The term "stage" in this and ensuing discussions is used arbitrarily to designate various steps in the development of the larva.

Serial sections of the intestine of infected beetles show that the embryos, within 14 hours after ingestion, already have initiated their penetration of the intestinal epithelium of the mid-gut (Fig. 1). In some cases the penetration has proceeded further, and they are now located between the epithelium and the muscular lining of the intestine (Fig. 2).

After 24 hours the embryos have reached the body cavity, and may be found either free in the hemocoel of the insect, or still partially in contact with the muscle layer of the intestine (Fig. 3).

Forty-eight hours after ingestion, the embryos are free in the hemocoel. At this point they differ little from the embryos within the eggs, measuring 0.039–0.042 mm. wide (across the hooks), and 0.039 mm. long. There is little cellular differentiation. The elliptical areas, mentioned above, are usually present and measure approximately 0.010 mm. in diameter (Fig. 4). This stage has been designated stage 1.

After 6 days within the insect the embryos begin a period of growth. There is no cellular differentiation, though the cells have increased in size and number. The hooks have retained more or less the same arrangement which they have when the embryo is still within the egg, i.e. arranged in three pairs. At this time there is the appearance of a cavity in the center of the embryo (Fig. 5). This is stage 2, which measures 0.054 by 0.068 mm.

Larvae 8–10 days old have doubled in size, and measure 0.110–0.150 by 0.120-

0.129 mm. The central cavity is very conspicuous, measuring approximately 0.044 by 0.034 mm. There is little cellular differentiation and the hooks are still arranged roughly in pairs. This is stage 3.

In larvae 11–12 days old the scolex and sucker primordia are discernible at the anterior end. The suckers appear as faintly outlined oval pads. Calcareous granules, absent in preceding stages, are distributed throughout the cytoplasm of the larva. At the posterior end of the larva there is a minute invagination. The granules and invagination are present in all succeeding stages. Rendtorff (1948) noted a similar invagination in the developing larvae of *O. symmetrica*. He observed the longitudinal excretory ducts of the larva emptying into a small bladder; the invagination marks the opening of a duct which appears to drain this bladder. The excretory ducts and bladder were not apparent in either stained or living larvae of *O. deserti*. In this, and all subsequent stages, the embryonic hooks are present in less orderly arrangement than in the onchosphere, usually scattered near the middle and posterior third of the larva. The larva has the general shape shown in Fig. 6, (calcareous granules and hooks not shown), being more or less elongate with the anterior end, or future scolex, slightly narrower than the body. The total length is 0.180–0.270 mm., width of the body 0.157–0.233 mm., the scolex primordium 0.135–0.180 mm. wide. This stage at which the suckers first become manifest is stage 4.

In 13 day old larvae the radial muscles of the suckers are distinct. The calcareous granules have increased in number; concentrated at the surface of the larvae, they are confined principally to the body. Only a few may be found in the scolex. The cavity present in preceeding stages has increased in size and occupies the central area of the body of the larva. A cyst wall, absent in younger stages, is now conspicuous, measuring 0.005–0.006 mm. in width. The calcareous granules, varying in size, measure up to 0.010 mm. The larvae are approximately the same size as in stage 4. This is stage 5 (Fig. 7; Fig. 17). Some of the larvae at this time have begun to invaginate the scolex.

It is of interest to note that the scolex is fully formed prior to invagination, whereas, in the development of the cysticercoids of certain other cestodes, the scolex primordium is first withdrawn and then differentiation proceeds within the vesicle containing the scolex (Hamann, 1889).

In approximately half of the larvae dissected from one beetle at 14–15 days, the scolex is in various degrees of invagination (Figs. 8 and 9; Fig. 18); the remainder of the larvae still have the scolex completely out. Stage 6 represents larvae with the scolex partly invaginated.

Larvae 16 days old have the scolex completely invaginated into the cavity which was formed early in the development of the larva. These are fully developed, infective cysticercoids, or stage 7. The actual process of invagination was not observed. In the mature cysticercoids (Figs. 19 and 20), calcareous granules are very numerous, obscuring most of the internal anatomy. The hooks are retained, but scattered, usually near the surface of the larva. The cyst wall is conspicuous, encircling the entire cysticercoid (Fig. 20). The invaginated scolex does not completely fill the cavity, leaving a narrow space between it and the body tissue (Fig. 20). At no time in the development is there any evidence of cercomere formation.

The early, fully-developed cysticercoids are quite mobile, undergoing peristalsis-

like contractions. After a few days movement ceases and the larvae assume a spherical shape.

At 16 days when the cysticercoid is fully formed, and thereafter, the larvae undergo no further change in size or development. The mature cysticercoids measure 0.135–0.187 mm. long by 0.150–0.195 mm. wide. The suckers are 0.051–0.076 mm. long. Mature cysticercoids 87 days within the insect are still infective as determined by feeding them to final hosts with the subsequent recovery of the adult tapeworm.

Rendtorff (1948) observed in beetles infected simultaneously with the cysticercoids of *O. symmetrica* and an undetermined species of gregarine, that although the cysticercoids were sometimes normal, more often they were shriveled and abnormal in appearance. The writer frequently obtained simultaneous infections of the cysticercoids and an unidentified gregarine in *Tribolium confusum* adults. The cysticercoids in all cases were of normal appearance. Rendtorff's findings were not investigated further by him, and their significance remains unknown.

Rendtorff also noted occasionally a thin membrane enclosing one or several cysticercoids taken from beetles. Cells were noted either lining the interior of the membrane or lying free in the space between the membrane and the cysticercoid. The cestode larvae were normal in appearance. The present writer, on several occasions, has observed a similar membrane enclosing the cysticercoid of *O. deserti* dissected from *T. confusum* adults. A small cluster of cells was noticed lying between the cysticercoid and the membrane, or lining the internal surface of the membrane. The cysticercoids appeared normal in all cases.

The origin or function of this membrane is not known. It seems likely that it is of host origin. Very little is known of the immune defenses, if any, of intermediate hosts to cestode larvae. This phenomenon would bear further investigation.

Definitive Hosts Infected

The first attempts to complete the life-cycle by infecting definitive hosts were carried out with cysticercoids of *O. deserti* dissected from the body cavity of the intermediate host. The larvae, in saline, were pipetted into the throat of the final host. In all cases at autopsy, no adult worms were found in the intestine of the host. A total of 15 kangaroo rats were used in these experiments.

During the dissection of the cystericoids from the body cavity of the beetles, it was noticed that the larvae became markedly brown, after a few minutes in saline exposed to the air. It was thought that the discoloration might be an oxidation, which in some manner affected the viability and infectivity of the cysticercoids. Hopkins (1952) observed a similar browning of the eggs and strobila of *Schistocephalus solidus* after exposure to elevated oxygen tensions *in vitro*.

Experiments were then carried out using lightly crushed infected beetles, which were introduced into the throat of the final host with forceps. At autopsy, adult tapeworms were recovered from the intestine of 2 of the 7 animals tried.

To test further the lack of infectivity of free cysticercoids, an experiment was set up using 11 kangaroo rats, six of which were fed infected beetles, and five fed free cysticercoids (15–50 cysticercoids per animal). At autopsy, all of the animals fed free cysticercoids were negative, whereas 3 of the 6 animals fed infected beetles harbored adult cestodes. In all subsquent experiments, therefore, the definitive

hosts were infected by feeding infected intermediate hosts. With this method, the size and number of cysticercoids fed to the animal could not be precisely determined. These data were estimated by dissecting beetles from the same culture as those used for infection experiments, but it was not always certain that the intermediate hosts fed to the animals actually contained cysticercoids. This may account for some of the negative results obtained using this method.

The results obtained by feeding infected beetles to definitive hosts (parasite-free as determined by fecal examination) is summarized in Table II.

Laboratory mice and rats could not be infected, although the kangaroo rats used as controls always were positive.

The small number of worms harbored by *Perognathus* is probably due to the small size of the animal (less than 160 mm.) compared to the much larger kangaroo rat and ground squirrel (over 200 mm.). No attempt was made to follow the development of the adult cestode in the final host. Gravid proglottids were formed and appeared in the feces 22 to 23 days after the ingestion of the infected intermediate host.

TABLE II.—*Results of feeding infected intermediate hosts experimentally to definitive hosts*

Species	Total no. tried	No. infected	Percentage infected	No. worms in each case
Albino rat or hooded rat	8	0		
Albino mouse	8	0		
Mesocricetus auratus	5	3	60	7 ; 6 ; 5
Dipodomys m. merriami	35	16	45	5 ; 1 ; 3 ; 11 ; 1 ; 10 ; 12 ; 14 ; 1 ; 3 ; 1 ; 2 ; 1 ; 12 ; 2
D. panamintinus mohavensis	11	7	64	1 ; 3 ; 3 ; 1 ; 4 ; 1 ; 1
Citellus leucurus	12	6	50	1 ; 1 ; 1 ; 1 ; 7 ; 4
Perognathus longimembris	10	3	30	1 ; 2 ; 1
Totals (for positive animals only)	73	35	48	

Effect of Temperature on the Development of the Cysticercoids

There are few reports in the literature of the effects of temperature on the development of the cysticercoids of anoplocephaloid cestodes. In general, at low temperatures, the cysticercoids require a longer time to attain maturity than at higher temperatures. Potemkina (1944a, 1944b) reported that *Moniezia benedeni* required 100 to 120 days to reach maturity at 26° C. and 140 to 150 days at 17° to 19° C., and that *Thysaniezia giardi* required 130 to 150 days to complete development at 17° to 19° C. Freeman (1952b) studied the effect of controlled temperatures on the larvae of *Monoecocestus americanus* and *M. variabilis* in oribatid mites. He found that as the temperature decreased the larvae required a correspondingly longer time to reach maturity. Rendtorff (1948) noticed that the cysticercoids of *Oochoristica symmetrica* attain maturity in nine days during warm weather, whereas, during cold months, 20 day cysticercoids were still immature. His is the only information previously available on the effect of temperature on the development of linstowiid larvae.

During the month of July, 1952, I obtained mature cysticercoids of *Oochoristica deserti* from the adults of *Tribolium confusum* in 27 days, at a relatively high room temperature. During the succeeding colder months (November 1952 to March 1953) the developmental rate was slowed considerably, so that as much as 92 days after infection of the insect, the cestode larvae were still immature.

It was decided, therefore, to test the effect of temperature under more precise conditions. Ninety-three *Tribolium confusum*, infected in the same manner, were divided into three groups. Group A was kept at 20° C., Group B at 26° C., and Group C at 30° C. Samples from each group were dissected periodically on identical days, and the stage of development and measurements of the larvae recorded.

The results are shown in Fig. 1. The stage of development (for explanation of stages see section on development in the intermediate host) is plotted against the number of days to reach that stage, at each of the three temperatures. The points plotted represent a particular stage found on the earliest day after feeding. Larvae at 30° C. reached maturity in 17 days. Those at 26° C. required a longer time, 20 days, to complete development. Forty-eight days after feeding the larvae at 20° C. had undergone only slight growth. These larvae were not followed further, but preliminary experiments suggested that they probably would have

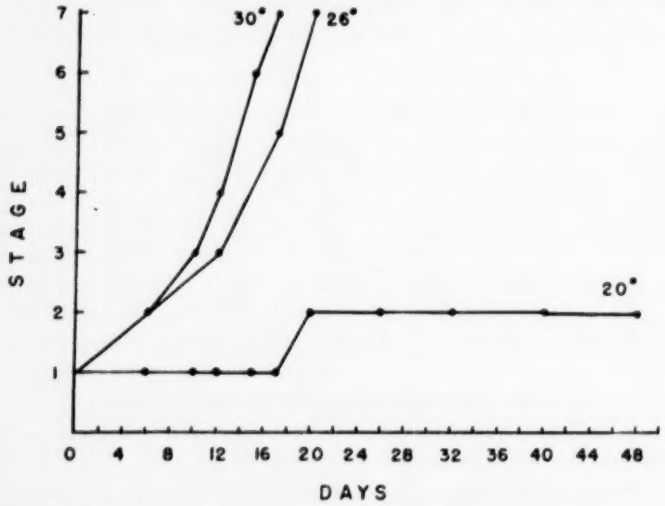

FIG. 1. The effect of temperature on the development of *Oochoristica deserti* in *Tribolium confusum*. Recognizable developmental forms from the onchosphere to the mature cysticercoid are represented by stages 1 through 7 (see text for descriptions).

completed development at 60 to 70 days. Freeman (1952b) obtained similar results with *Monoecocestus* cysticercoids in mites. The larvae of this cestode, at 25° C., 20° C., 15° C., required 45, 52, and 81 days, respectively, to reach maturity. The results indicate that temperature is an important factor in determining the developmental rate of anoplocephaloid cestodes, possibly of cestodes in general. No doubt temperature is of considerable importance in nature as a factor in the mechanics of the life-cycle of the cestode. This is particularly true in the case of *Oochoristica deserti* since large daily and seasonal temperature changes occur in the desert. In one experiment, the writer transferred infected beetles, after one month at room temperature, to 28° C. The rate of larval development was noticeably increased. Although it would have been more desirable to have kept the insects at an initial lower temperature, this at least suggests that the larvae can survive low temperatures, and that perhaps in nature they are able to overwinter in the intermediate host.

Freeman (1952b) noted that with a decrease in temperature, the average size of the mature cysticercoids of *Monoecocestus* increased. He suggested that *Monoecocestus* is a cestode which follows Bergmann's rule, which states that the same species, having a north to south distribution, will be smaller in warm climates and larger in cold climates. The present writer questions the applicability of Bergmann's rule to cestodes on the basis that the rule is primarily concerned with warm-blooded animals (it correlates the surface-volume ratio of the animal with their heat conservation) and not with cold-bloods. In the case of cold-blooded animals it is difficult to make a general statement. Some, particularly terrestrial animals, attain their largest size in warmer, rather than in colder climates (Allee *et al.*, 1949).

With *O. deserti*, the writer found no significant differences in the size of the mature cysticercoids at the three temperatures studied.

DISCUSSION

The experiments on the life-cycle of *Oochoristica deserti* showed that members of the orders COLEOPTERA and LEPIDOPTERA were capable of serving as intermediate hosts.

No attempts were made to discover the intermediate hosts of this tapeworm in nature. Thus, one can only speculate on the natural life-cycle of this parasite. It is of interest that the larval stages of the intermediate host may be experimentally infected, and that through holometabolous metamorphosis of the insect the cysticercoids are transferred to, and remain infective in, the adult insect. Such an ability may aid in the distribution of the parasite, as is the case with plagiorchid trematodes and certain spiruroid nematodes. The distribution may be effected if the adult insect and not its larval stage, inhabits the same ecological niche as the final host, or if the adult insect is a more acceptable food item for the definitive host than the larval insect. The latter possibility would seem to be more applicable to coleopterans and desert rodents. Read and Millemann (1953) in a study of the helminths of kangaroo rats found three species of nematodes, two of which are known, and the third suspected, to use various insects as intermediate hosts. These findings, together with the facts of the *Oochoristica* life-cycle, support the idea that kangaroo rats are not exclusively seed eaters. These animals are already known to eat occasional grasshoppers and other insects. It would seem then that, at least during certain times of the year, perhaps when suitable plant material is scarce, kangaroo rats actively seek out and feed upon terrestrial, nocturnal insects.

Insects, including tenebrionid coleopterans, are present in the desert during certain times of the year, some during the entire year. Tenebrionids and dermestids typically are nocturnal and feed on decaying plant and animal material. It is reasonable to believe that these insects and possibly others may feed on, or accidentally ingest, fecal material containing the tapeworm ova. After a suitable period, such insects would be infective for the kangaroo rat.

Although *Dipodomys m. merriami, D. panamintinus mohavensis, Perognathus,* and *Citellus* may be experimentally infected, only *Citellus* and *Dipodomys m. merriami* have been found naturally infected with the adult tapeworm. Since these animals occupy the same ecological niche, the food habits of the animals, therefore, may be the decisive factors in the maintenance of natural infections. In

the case of *Perognathus*, the natural intermediate host may be too large to be eaten by this small animal.

The studies of Rendtorff (1948), of Millemann and Read (1953) on *O. scelopori*, and the one reported here for *O. deserti*, bring to three the number of life-cycles known in linstowiid cestodes. The fact that this group of cestodes uses insects as the intermediate hosts, and that their larvae fail to develop in mites, furnishes further justification for their separation as a family distinct from the ANOPLOCEPHALIDAE.

The known experimental intermediate hosts for the three species of *Oochoristica* are summarized in Table III which also lists the insects experimentally capable of serving as intermediate hosts, and those which failed to become infected. It can be seen that these cestodes show little specificity with regard to the intermediate host in which they may develop. It seems likely that further study will extend the list.

TABLE III.—*Known experimental intermediate hosts for* Oochoristica *spp.*

Intermediate host	*O. symmetrica*	*O. deserti*	*O. scelopori*
Trogoderma versicolor	+		
Attagenus piceus	+	0	0
Tribolium confusum	+	+	+
T. ferrugineum	+		
Anthrenus verbasci	+		
Tenebroides mauritanicus	+		
Tenebrio molitor	0	+	0
Plodia interpunctella	+		
Gnathocerus cornutus		+	
Ephestia cautella		+	
Dermestes maculatus		+	
Undetermined sp. of beetle	+		

SUMMARY

1. A new species of cestode, *Oochoristica deserti*, from *Dipodomys m. merriami* and *Citellus leucurus* is described.

2. The experimental life-cycle of *Oochoristica deserti* has been determined. The cysticercoids develop in the adult and larval stages of various coleopteran and lepidopteran insects, but not in acarid mites. Cysticercoids mature in 16 days in the insect at 30° C., and remain infectious for at least 87 days. *Dipodomys m. merriami, D. panamintinus mohavensis, Citellus leucurus, Perognathus longimembris* and *Mesocricetus auratus* were experimentally infected with the cysticercoids of *O. deserti*. Gravid proglottids were formed and passed 22–23 days after ingestion of the cysticercoids. Attempts to infect laboratory rats and mice were unsuccessful.

3. Penetration glands are present in the onchosphere of *O. deserti*.

4. Study of the effect of temperature on the development of the cysticercoids showed that as the temperature decreases the cestode larvae require a correspondingly longer time to attain maturity.

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EXPLANATION OF PLATES

PLATE I

Developmental forms of *Oochoristica deserti* n. sp.

FIGS. 1–3. Stages in the penetration of the embryo through the intestine of the insect.

FIG. 4. Embryo from the hemocoel showing "elliptical areas". Stage 1.

FIG. 5. Formation of central cavity. Stage 2.

FIG. 6. Appearance of scolex and sucker primordia. Stage 4.

FIG. 7. Suckers well-developed. Stage 5.

FIGS. 8 & 9. Beginning invagination. Stage 6.

PLATE II

Oochoristica deserti n. sp.

FIG. 10. X-section through testes and yolk gland region of mature proglottid showing parenchymal musculature in two layers.

FIG. 11. Scolex.

FIG. 12. Egg.

FIG. 13. Embryonic hook.

FIG. 14. Mature proglottid.

FIG. 15. Penetration glands in the embryo.

FIG. 16. Penetration glands in the embryo of *Raillietina cesticillus* (from Reid, 1948).

PLATE I

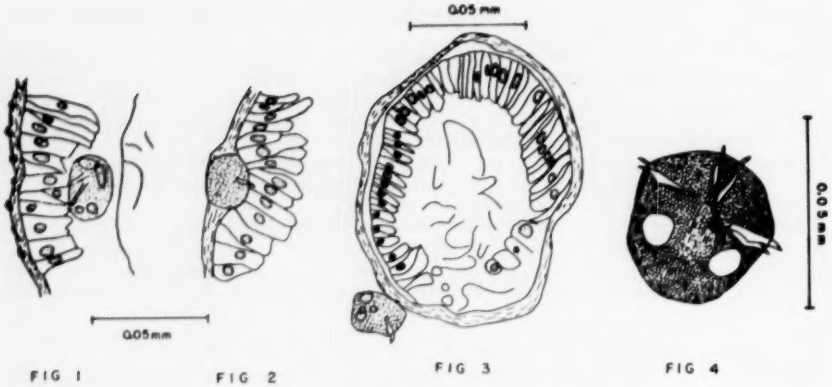

FIG 1 FIG 2 FIG 3 FIG 4

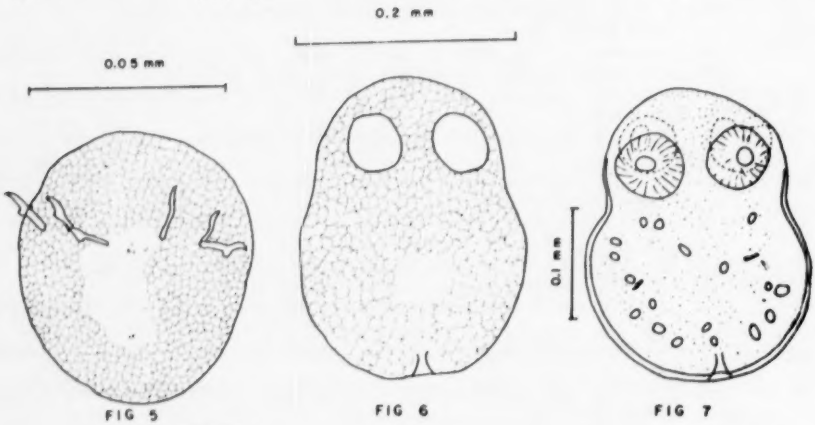

FIG 5 FIG 6 FIG 7

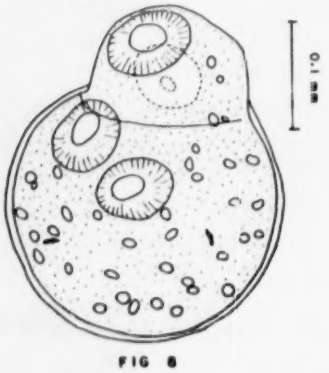

FIG 8 FIG 9

PLATE 2

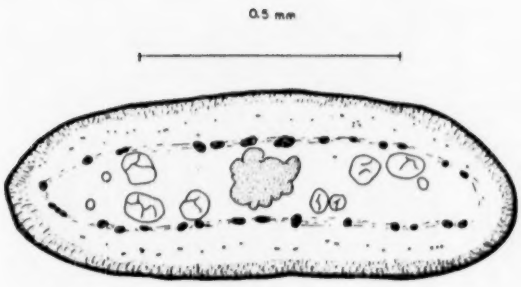

FIG 10

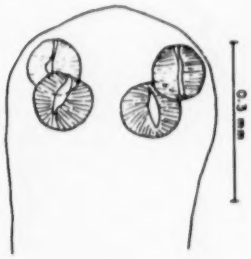

FIG 11

FIG 12

FIG 13

FIG 15

FIG 16

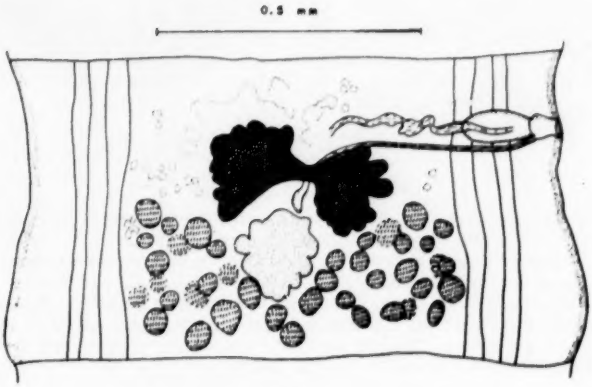

FIG 14

PLATE III

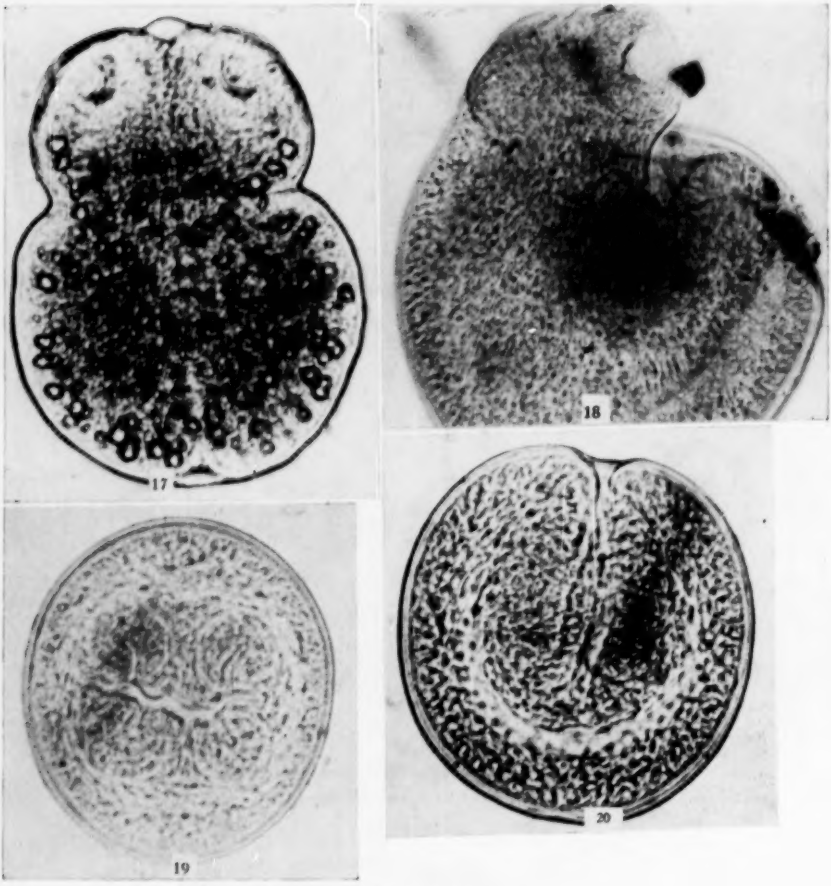

PLATE III

Oochoristica deserti n. sp.

FIG. 17. Photomicrograph. Living material showing calcareous granuales, minute posterior invagination, and well-developed suckers. Stage 5. (Approx. 340×).

FIG. 18. Photomicrograph. Stained material. Calcareous granules not shown. Beginning invagination. Stage 6. (Approx. 450×).

FIG. 19. Photomicrograph. Stained material. Calcareous granules not shown. Mature cysticercoid. Stage 7. Polar view. (Approx. 380×).

FIG. 20. Photomicrograph. Stained material. Calcareous granules not shown. Mature cysticercoid. Stage 7. Lateral view. (Approx. 380×).

RESEARCH NOTES

TAXONOMY OF SOME DIGENETIC TREMATODES FROM STURGEONS

Wherever sturgeons occur, they evidently harbor a distinct group of trematodes belonging to at least three genera, *Dercpristis, Pristicola* and a third for which it is now evident that two names have been proposed, *Pristotrema* and *Skrjabinopsolus*. *Pristotrema* was proposed by the writer (Cable, 1952, Parasit. **42:** 85–91) for a species in sturgeon of the Wabash River, U.S.A. For *Skrjabinopsolus*, a genus erected for a sturgeon parasite in Russia, the exact author and date are less certain. Bychowsky and Dubinina (1954, Zool. Zhur. **33:** 788–793) give Ivanov, 1937, but the paper cited is by Ivanov and Murygin (1937, Skrjabin Jubilee Vol., 253–268) who name Ivanov as the authority for *Skrjabinopsolus* with 1934 as the date in one place and 1935 in another. Reference to a paper of either date is not given and the writer has been unable to find a characterization of the genus dated prior to 1937. Unless such a diagnosis has been overlooked, the correct designation is *Skrjabinopsolus* Ivanov in Ivanov and Murygin, 1937. *Skrjabinopsolus* thus has priority over *Pristotrema* which is hereby suppressed; *P. manteri* thus becomes *Skrjabinopsolus manteri* (Cable, 1952) comb. nov.

Molin (1861, Denkschr. kaiserl. Akad. Wissensch. Wien. Math.-Naturw. Cl. **19,** Abt. 2, 189–338) supplemented descriptions of two species which, although inadequately characterized, obviously belong to the group under discussion. They are *Distomum semiarmatum* which probably is a species of *Skrjabinopsolus* and *D. ellipticum* which possesses the expanded forebody of *Dercpristis* but evidently lacks the enlarged spines typical of that genus. Cable and Hunninen (1942, Biol. Bull. **82:** 292–312) observed that in *Dercpristis inflata*, the enlarged spines of the cervical expansions and dorsal hump were part of, and blended into, the general body spination. Localized enlargement of spines, therefore, may be a matter of degree rather than an absolute one from species to species of the genus *Dercpristis*.

In assigning these trematodes to the ACANTHOCOLPIDAE, Bychowsky and Dubinina (*loc. cit.*) have disregarded recent studies dealing with life histories and the significance of differences in adult morphology as well. Caballero (1952, Rev. Med. Vet. Parasit. **11:** 1–231) has excluded these parasites from that family and in papers cited above, the writer has presented evidence of a closer relationship to the LEPOCREADIIDAE than to the ACANTHOCOLPIDAE. Actually, Bychowsky and Dubinina have added significantly to that evidence in reporting from a silurid fish a new genus, *Paratormopsolus*, which combines features of the DEROPRISTIINAE (scanty vitellaria, posterior extent of uterus) and "typical" lepocreadiids (external seminal vesicle, lack of cirrus and metratermal spines, absence of conspicuous body spination).

One of several constant differences between the ACANTHOCOLPIDAE and the DEROPRISTIINAE concerns storage of sperms in the female complex. In true acanthocolpids, the beginning of the uterus serves that purpose whereas in lepocreadiids, there is a separate seminal receptacle which is especially prominent in the DEROPRISTIINAE. Ivanov and Murygin (*loc. cit.*) described such a receptacle as a specific character of *Skrjabinopsolus acipenseris* but omitted that highly important feature from the generic diagnosis. Bychowsky and Dubinina (*loc. cit.*) evidently missed the receptacle completely in *S. acipenseris* and probably did so in *Paratormopsolus* as well.—R. M. CABLE, *Purdue University, Lafayette, Indiana.*

PARASITES FROM COATI (*NASUA NARICA*)

On February 10, 1952, an adult coati (*Nasua narica*) was taken by the Walla Walla College Museum of Natural History Field Expedition to Southern Mexico. The specimen was collected by the writer along the Christopher Columbus Highway, only 10 miles north of Arriaga, Chiapas, near the border of the states of Oaxaca and Chiapas. Examination of the digestive tract revealed three species of parasites: 11 nematodes, *Physaloptera maxillaris*, from the stomach; 53 acanthocephalans, *Prosthenorchis lühei*, from the large intestines; and two specimens of *Trichurus* sp. from the large intestine about four cm. from the anus. This is probably a new host record for *Trichurus*. The species of *Trichurus* could not be determined since both specimens were females. Curatella fruit was the only food found in the digestive tract of the coati. Dr. Asa C. Chandler determined the parasites.—ERNEST S. BOOTH, *Walla Walla College.*

A COLLECTION OF FLEAS FROM THE VICINITY OF FORT SIMPSON, NORTHWEST TERRITORIES, CANADA

In 1954 Mr. P. M. Mann presented to the Department of Zoology, University of Western Ontario, a small collection of fleas taken by himself from mammals during the course of biological studies in the vicinity of Fort Simpson, Northwest Territories, Canada. The specimens have been mounted individually in Canada balsam on microscope slides and the slides have

been incorporated into the collection of entomological slide mounts in the Department of Zoology, University of Western Ontario. In the following account the catalogue numbers of the slides are included among the data pertaining to the specimens. The fleas were identified with keys in G. P. Holland (1949, The Siphonaptera of Canada. Tech. Bull. 70, Dominion Canada Dept. Agric., 306 pp.). Mr. Holland, Head of the Systematic Entomology Unit, Department of Agriculture, Ottawa, kindly examined the slides and checked the identifications. Each numbered host listed below is based upon a collection from a single adult mammal except in the case of *Tamiasciurus hudsonicus* in which case the fleas were removed from suckling squirrels taken from a nest.

1. *Clethrionomys dawsoni* Merriam (Fort Simpson, N.W.T., July 12, 1953): *Epitedia wenmanni* (Rothschild)—1 male (slide E19.38); *Megabothris quirini* (Rothschild)—1 female (E19.39).

2. *Clethrionomys dawsoni* Merriam (Fort Simpson, N.W.T., July 15, 1953): *Megabothris quirini* (Rothschild)—2 males, 1 female (E19.40–42).

3. *Peromyscus maniculatus borealis* Mearns (Nahanni Butte, at the confluence of the South Nahanni and Liard Rivers, N.W.T., August 2, 1953): *Orchopeas leucopus* (Baker)—4 males, 4 females (E19.43–50).

4. *Tamiasciurus hudsonicus* subsp., four suckling squirrels removed from a nest, (Keele River, 27 miles upstream from its confluence with the Mackenzie River, June 17, 1953, D. R. Flook and P. M. Mann): *Orchopeas caedens* (Jordan)—3 females (E19.51–53); *Monopsyllus vison* (Baker)—11 males, 18 females (E19.54–82).—W. W. JUDD, *Department of Zoology, University of Western Ontario, London, Ontario.*

NOTES ON COLLECTING TREMATODE-INFECTED AMNICOLIDAE IN DEEP WATERS

In an ecological study Eggleton (1952, Tr. Am. Micr. Soc. **71**: 189–228) reported that 75 percent of the total gastropod population in the interdepression area of Douglas Lake, Michigan consisted of Amnicolid snails. This area is an expanse of open water about 1.5 square miles totally devoid of surface vegetation but not exceeding 20 feet in depth. No examination of AMNICOLIDAE for trematode cercariae had been made from waters in this area largely because the middle of a lake is an unlikely place to look for snails or to attempt to collect them by ordinary means. Yet it became apparent from extensive collecting along the shores that both vegetation and snails were more abundant in waters just out of reach of a wader's dip net.

The tentative idea that the interdepression area might serve as a source of snails harboring cercaria prompted me to test this assumption. The first collection obtained with a Reighard tow net consisted of 420 snails, three infected. Though further collecting disclosed a mere one percent infection rate this rate was significant when compared with a two percent rate for near-shore collections. The use of aqua lung diving equipment disclosed extensive patches of submerged vegetation and large numbers of snails as well as fish which could serve as trematode hosts. The minimum requirements for maintaining infections in this region of the lake were explained.

The Reighard net consists of a fine mesh cone net suspended from a triangle-shaped serrated steel rim. Three rods extending the length of the net serve as runners. This net proved to be very effective for collecting snails when towed through vegetation in deep water along the lake's shelf. On several occasions large numbers of snails were found in such deeper areas when vegetation in shallow waters was negative. It appears that AMNICOLIDAE tend to occupy the cooler, deeper habitats during the warm summer months. A number of cercarial species was found in Amnicolids in such areas as well as the interdepression area. It thus seems reasonable to assume that comparable collecting techniques might be fruitful on other inland lakes where Amnicolid snails are sought for trematode life history studies. This is contribution #71 from the Department of Natural Science, Michigan State College and the University of Michigan Biological Station.—WALTER S. LUNDAHL, *Michigan State College.*

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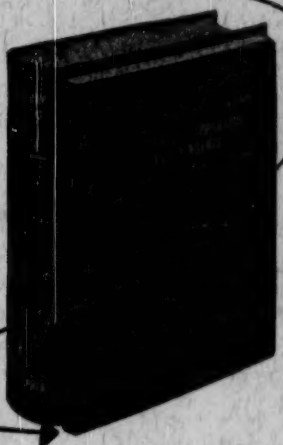

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